IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	CEBON, et al.
Title:	In Vivo Efficacy of NY-ESO-1 Plus Adjuvant
Appl. No.:	10/573,753
International Filing Date:	9/30/2004
Examiner:	Marianne Dibrino
Art Unit:	1644
Confirmation Number:	3988

RESPONSE TO REQUEST FOR INFORMATION UNDER 37 CFR 1.105

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This is a response to the Request for Information set forth in the Advisory Action mailed July 12, 2010, in the captioned patent application. As this is the first response to the Request for Information, the fee and certification requirements for the documents submitted herewith (listed on the accompanying Form PTO/SB/08) are waived, as stated at page 3 of the Advisory Action. Applicant respectfully requests that each listed document be considered by the Examiner and be made of record in the present application and that an initialed copy of Form PTO/SB/08 be returned in accordance with MPEP §609.

RESPONSE TO REQUEST FOR INFORMATION

The Patent Office has made a Request for Information related to the Cebon abstract presented at the 2002 annual meeting of the American Society of Clinical Oncology (the "Cebon abstract"). In response, Applicant provides the following information, which is based on information received from Dr. Johnathan Cebon, an inventor of the application and coauthor of the abstract, slides and poster from the ASCO 2002 annual meeting.

The Cebon abstract was part of a poster displayed at the ASCO 2002 annual meeting, which was held in Orlando, Florida. A copy of the poster is submitted herewith as part of the response to the Request for Information. There was no oral presentation of the poster, abstract or underlying subject matter at the ASCO 2002 annual meeting. The slides were part of a "virtual meeting" accessible via the ASCO website to virtual meeting registrants.

Specific answers to the questions set forth in the Request for Information are provided below.

- **A statement describing the amount of ISCOM adjuvant in each of the different protein dosage administrations, i.e., for 10 ug, 30 ug and 100 ug of NY-ESO-1.**

The in vivo trial presented in the Cebon abstract and slides corresponds to that described in Example 1 of the application. Thus, the amount of protein and adjuvant used were:

Dose level A = 10 μg NY-ESO-1 protein in 12 μg ISCOM

Dose level B = 30 μg of NY-ESO-1 protein in 36 μg ISCOM

Dose level C = 100 μg of NY-ESO-1 protein in 120 μg ISCOM

Dose level D = 100 μg NY-ESO-1 protein without ISCOM

The specification as filed includes a clerical error with regard to dose level B, in that paragraph [0023] states that 36 μg protein was used, where it was 30 μg protein. The correct protein dose is indicated in Figures 1 and 2. This error is being corrected in the response submitted herewith.

- **A statement describing if reducing the risk of relapse was presented/discussed during the slide presentation.**

As noted above, there was no oral presentation or discussion of the subject matter underlying the Cebon abstract and slides at the ASCO 2002 annual meeting. Moreover, relapse data were not available at the time. Thus, there was no presentation of relapse data at the ASCO 2002 annual meeting.

- **A statement describing all of the data that was presented and how that data is related to the data of the instant specification.**

All of the data presented at the ASCO 2002 annual meeting are set forth in the Cebon abstract and slides already of record, and the poster submitted herewith (which is cumulative of the abstract and slides). As noted above, the in vivo trial presented in the Cebon abstract corresponds to that described in Example 1 of the application. Other data presented correspond to Example 2, Example 3 (Figure 2 but not Figure 3) and Example 4 of the application. The results reported in Examples 5-17 of the specification were not presented at the ASCO 2002 annual meeting.

- **In response to this request, Applicant is also requested to furnish:**

  A statement describing additional presentations and/or abstracts presented by Applicant at scientific meetings wherein data pertinent to the subject matter was disclosed, and the contents of such disclosures, if such disclosures in fact occurred.

There were two additional presentations of subject matter presented at the ASCO 2002 annual meeting and disclosed in the application, prior to the September 30, 2004 filing date of the PCT application. The first was a presentation at the December 2002 Australian Society of Immunology. A copy of the slides from that presentation are submitted herewith. The second was an invited seminar given at Auckland University in July 2003. A copy of the slides from that seminar are submitted herewith. These presentations include some additional data beyond that presented at the ASCO 2002 annual meeting, relating to CD4 and CD8 T

cell responses, the use of DCs to generate T cells, and immunohistochemistry experiments. However, again, no relapse data or patient survival data were presented.

As this response replies to each requirement for information giving the information required, it is believed to be a complete response to the Requirement for Information under 37 CFR 1.105.

Respectfully submitted,

Date August 2, 2010

By _____

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PTO/SB/08 (09-06)
Approved for use through 03/31/2007. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO				***Complete if Known***	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				**Application Number**	10/573,753
Date Submitted: August 2, 2010				**Filing Date**	9/30/2004
				First Named Inventor	Jonathan CEBON
(use as many sheets as necessary)				Art Unit	1644
				Examiner Name	Marianne Dibrino
Sheet	1	of	1	Attorney Docket Number	029860-0145

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number / Number-Kind Code[2] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

UNPUBLISHED U.S. PATENT APPLICATION DOCUMENTS

Examiner Initials*	Cite No.[1]	U.S. Patent Application Document / Serial Number-Kind Code[2] (*if known*)	Filing Date of Cited Document MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document / Country Code[3]-Number[4]-Kind Code[5] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Documents	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.) date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[6]
	A1	CEBON ET AL., "A phase I study of NY-ESO-1 ISCOM® in patients with NY-ESO-1 positive cancers and minimal residual disease," from ASCO Annual Meeting, 2002, Orlando, Florida (Poster).	
	A2	CEBON ET AL., "A phase I study of NY-ESO-1 ISCOM® in patients with NY-ESO-1 positive cancers and minimal residual disease," slides presented at Australian Society of Immunology, December 2002.	
	A3	CEBON ET AL., "Cancer Vaccination," slides presented at seminar given at Auckland University, July 2003.	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. 3 Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). 4 For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 5 Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. 6 Applicant is to place a check mark here if English language Translation is attached.

A phase I study of NY-ESO-1 ISCOM® in patients with NY-ESO-1 positive cancers and minimal residual disease

LUDWIG INSTITUTE FOR CANCER RESEARCH

CSL LIMITED

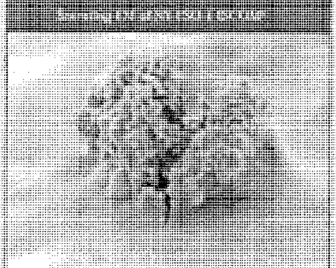
ABSTRACT

NY-ESO-1 is a cancer testis (CT) antigen expressed in a variety of tumors including melanoma, breast, lung, bladder, head & neck cancer, malignant myeloma, and synovial sarcoma. ISCOM®(Immunostimulating COMplex) are saponin-based adjuvants known to stimulate antibody responses and induce T helper cell as well as cytotoxic T lymphocyte responses in a variety of animal models and human clinical trials. The objectives of this study were to evaluate the safety and immunogenicity of an NY-ESO-1 ISCOM® vaccine. 46 patients (pts) with MRD and NY-ESO-1 positive tumors (immunohistochemistry or RT-PCR) received NY-ESO-1 ISCOM® or protein alone, administered IM at monthly intervals X 3. NY-ESO-1 ISCOM® protein doses were: 10ug (n=3), 30ug (n=3), 100ug (n=20). 20 patients received 100ug NY-ESO-1 protein alone. All pts except 1 in each of the 100ug groups were HLA-A2+. Pts were evaluated for toxicity weekly. Immune function was assessed by Delayed Type Hypersensitivity (DTH), antibody titre (ELISA), tetramer and enumeration of γ-interferon producing CD8+ T cells. Antigen specific T cells were enumerated in HLA-A2+ patients with assays using synthetic peptides in tetramers or on target cells. Tetramers were based on immunodominant sequences restricted by HLA-A2. Results: Grade 2 injection site pain, flu-like symptoms, fever and malaise were noted but well tolerated. Grade 3 injection site pain occurred in three pts who received 100ug. At the 100ug dose of NY-ESO-1 ISCOM® enhanced DTH reactions (up to 68mm redness and 28mm induration) over baseline occurred. Antibody titres >1:100,000 and NY-ESO-1 specific T cells were commonly seen. In Conclusion: vaccination with NY-ESO-1 ISCOM® was safe and high titre humoral and cellular immune responses to NY-ESO-1 occurred. Correlation with clinical response is required in patients with evaluable tumors.

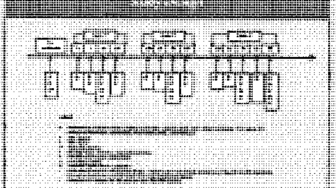
mRNA expression: NY-ESO-1

Tumour Type	%	n
Melanoma	41	144
Melanoma cell lines	33	30
Ca breast	40	25
HNSCC	20	10
TCC	25	4
Ca prostate	13	8
Hepatoma	47	31

Scanning EM of NY-ESO-1 ISCOM



Study Design



NUMBER OF PATIENTS

- Total: 46
- 3 parts
  - 1: NY-ESO-1/ISCOM®
    - 3 pts/cohort
    - Dose levels: A 10ug & B 30ug
    - Only HLA A2+ patients for purposes of immunological assays
  - 2: NY-ESO-1/ISCOM®
    - Dose 100ug expanded to 20 patients
    - 10 HLA A2+ve (2 placebo), 10 HLA A2-ve (2 placebo)
  - 3: Protein alone
    - 100ug expanded to 20 patients
    - 10 HLA A2+ve (2 placebo), 10 HLA A2-ve (2 placebo)

ASSAYS

- DTH using NY-ESO-1 protein alone
- Antibody (capture ELISA)
- CD8+ T cells
  - Tetramer: SLLMWITQC
  - Cytospot: γIFN producing CD8+T Cells
- ELISpot (similarly performed on some cohort A pts)
- Assays under development
  - CD4+ T cells (proliferation, cytokine secretion)
  - Class I epitopes - non HLA-A2

TOXICITY

- NY-ESO-1 ISCOM® were well tolerated
- Most adverse events were grade 1 or 2
- Grade 3 toxicities: injection site pain (x 3/46)
- Common grade 2 toxicities (2 or more patients)
  - Injection site pain
  - Fever
  - Myalgia
  - Headache
  - Flu-like symptoms

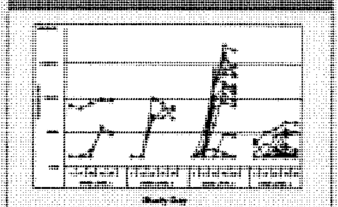
RESULTS

Cancer Types

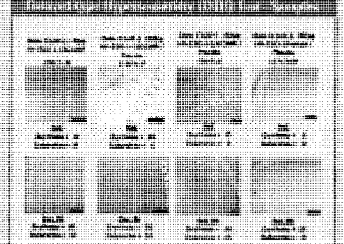
Ca. Study	[illegible]
Melanoma*	[illegible]
Ca Breast	[illegible]
Ca Ovary	[illegible]
TCC Bladder	[illegible]
Adrenal cortical carcinoma	[illegible]

*Stage II, III and IV resected

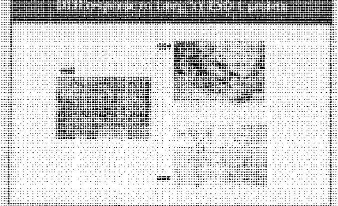
Antibody response to NY-ESO-1



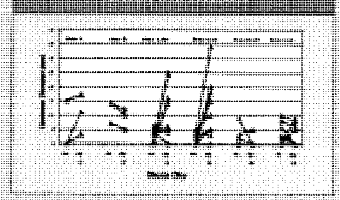
Delayed Type Hypersensitivity (DTH) Test – Samples

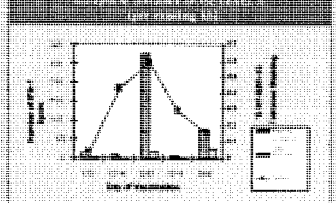


DTH response to 10ug NY-ESO-1 protein

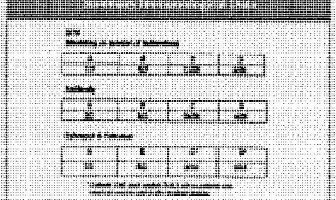


RESULTS (Continued...)





Summary Immunological Data



CONCLUSIONS

- NY-ESO-1 ISCOM® vaccinations were safely tolerated
- NY-ESO-1 ISCOM® generated both humoral & cellular responses
- ISCOM® adjuvant generated superior DTH and antibody responses
- Cytospot assay in HLA A2+ve patients: positive in 1 level A pt (with prior Ab response), 3 level C patients and 1 level D patients (in both levels C & D, 8 pts received NY-ESO-1 and 2 received placebo)
- There was a good correlation between tetramer & cytospot data
- Assays for other epitopes are pending however clear evidence of CD4 responses in few level C patient tested (further characterisation is underway)

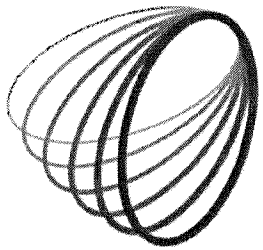
Austin & Repatriation Medical Centre

CDCT

LUDWIG INSTITUTE FOR CANCER RESEARCH

CSL LIMITED

A phase I study of NY-ESO-1 ISCOM® in patients with NY-ESO-1 positive cancers and minimal residual disease

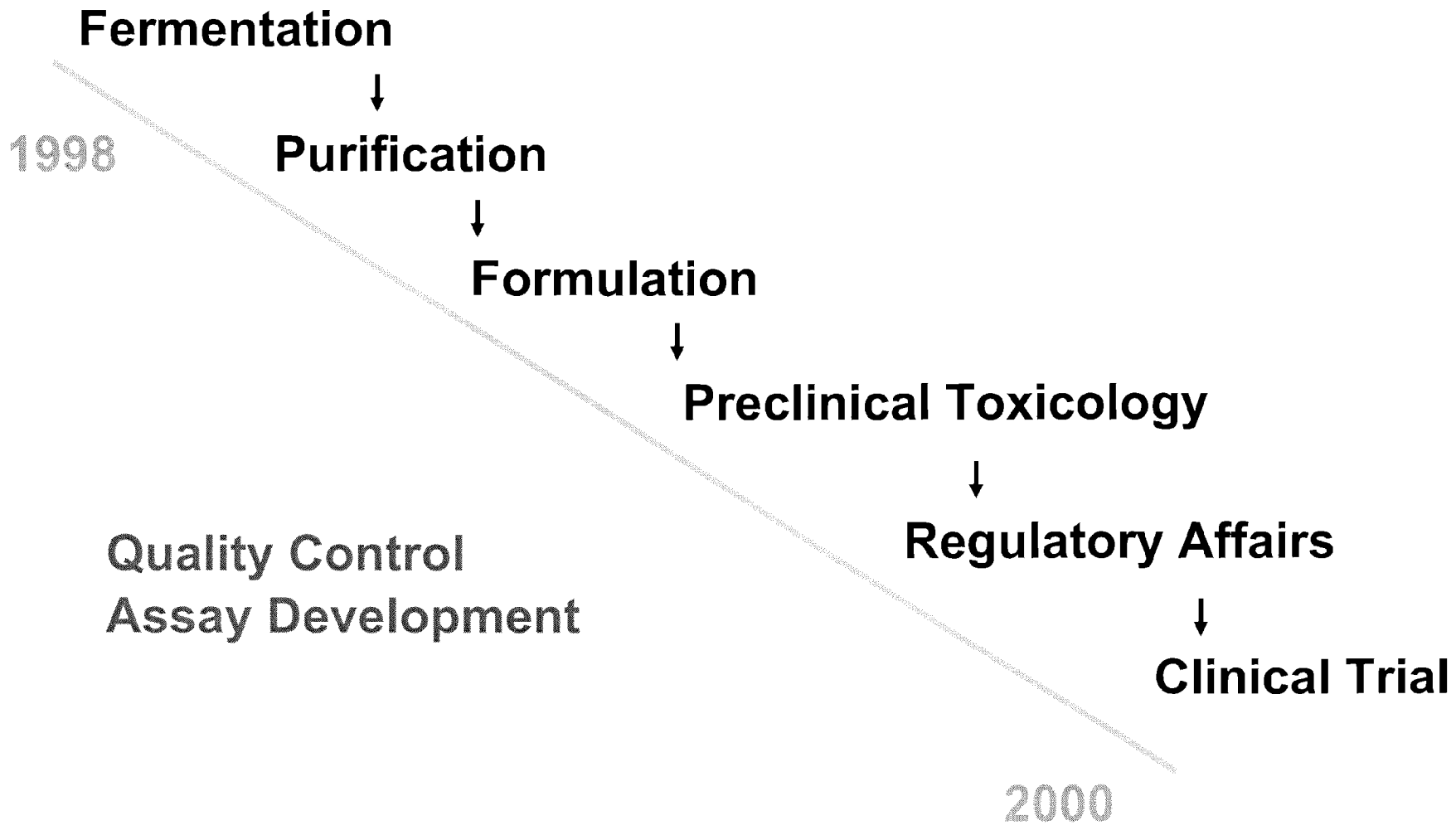


CDCT

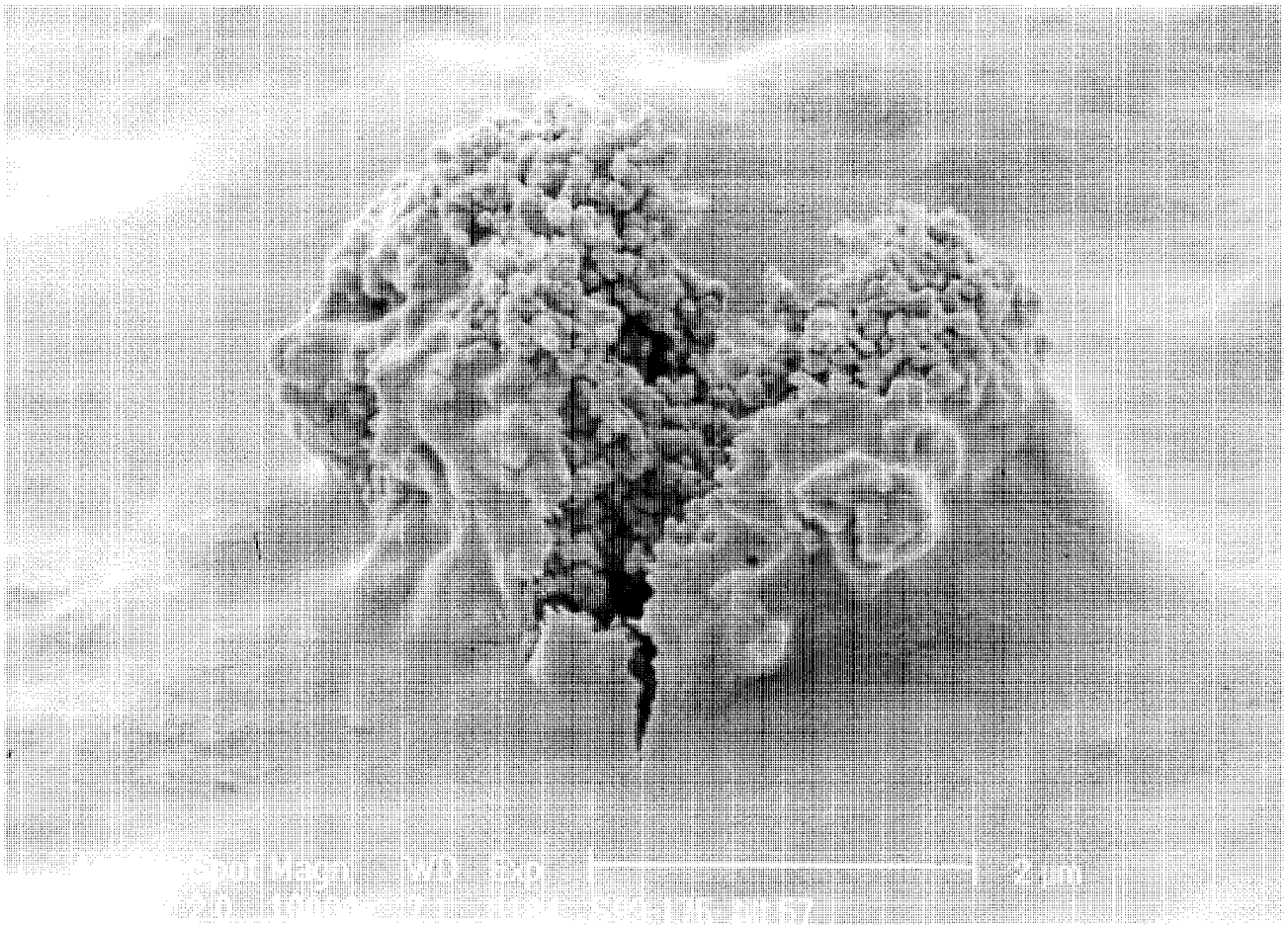
mRNA expression NY-ESO-1

Tumour Type	%	n
Melanoma	41	154
Melanoma cell lines	33	30
Ca breast	40	25
HNSCC	20	10
TCC	25	4
Ca prostate	13	8
Hepatoma	47	31

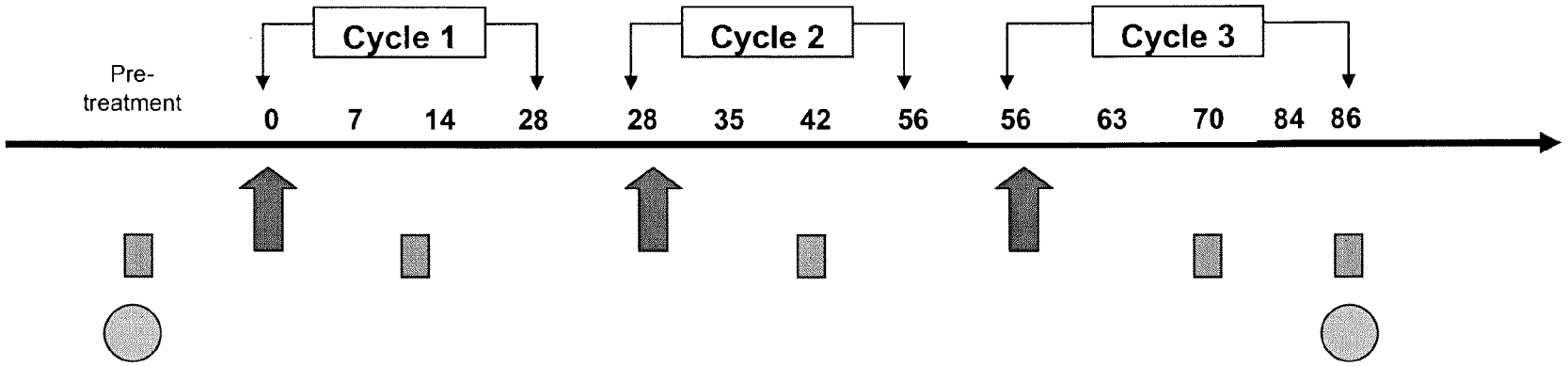
Vaccine Production Timeline



Scanning EM of NY-ESO-1 ISCOM®



Study Design



Vaccination

DTH

T-cell assays & serology

Patients

- Total 46
- 3 parts
  - 1 NY-ESO-1/ISCOM®
    - 3 pts/cohort
    - Dose levels A 10ug & B 30ug
    - Only HLA A2+ patients for purposes of immunological assays
  - 2 NY-ESO-1/ISCOM® - dose level C
    - Dose 100ug expanded to 20 patients
    - 10 HLA A2+ve (2 placebo), 10 HLA A2-ve (2 placebo)
  - 3 Protein alone - dose level D
    - 100ug expanded to 20 patients
    - 10 HLA A2+ve (2 placebo), 10 HLA A2-ve (2 placebo)

Cancer Types

On Study	**51**
Melanoma*	**46**
Ca Breast	**3**
TCC Bladder	**1**
Adenoid cystic carcinoma	**1**

*Stage II, III and IV resected

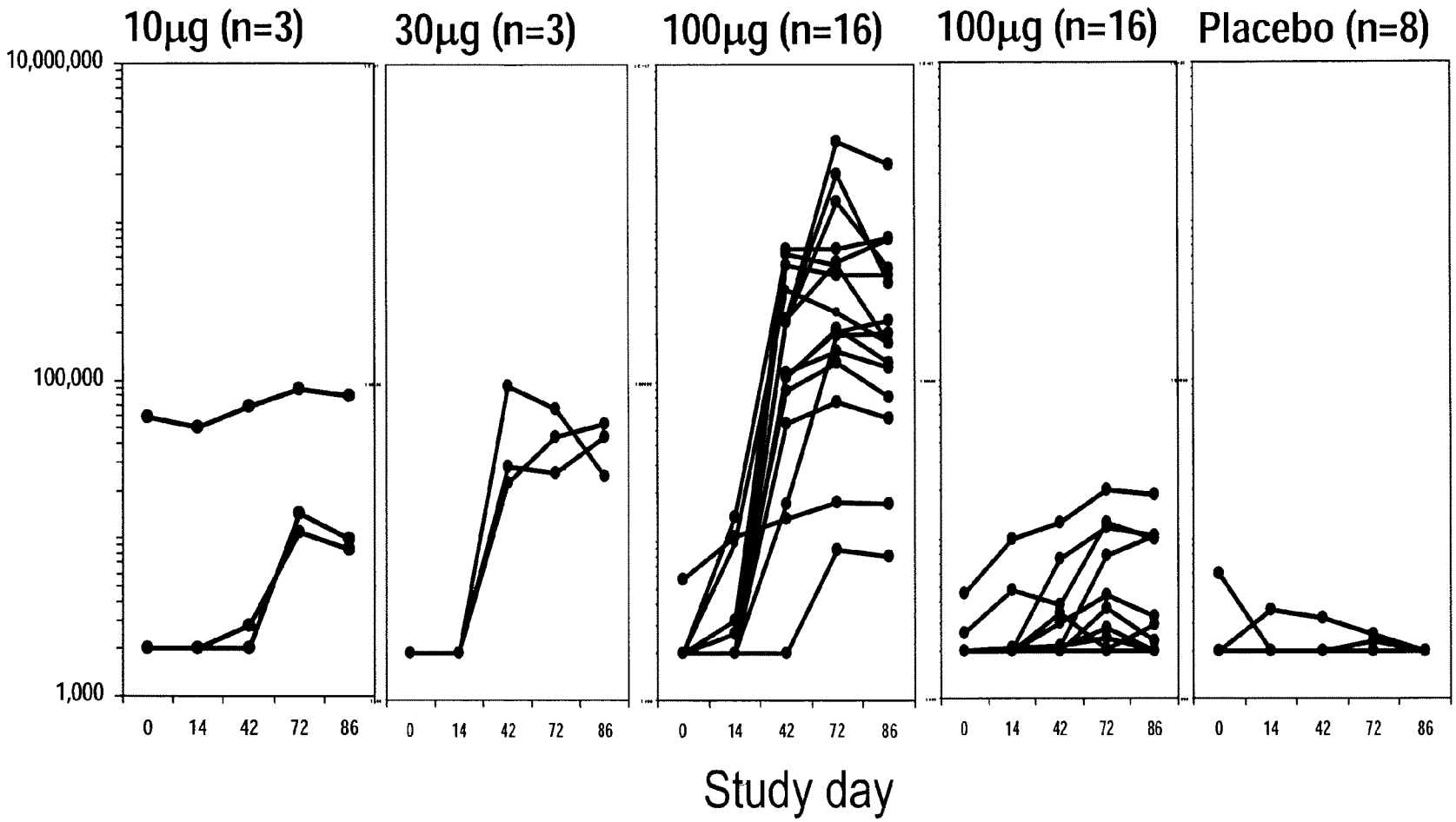
Toxicity

- NY-ESO-1 ISCOM® was well tolerated
- Most adverse events were grade 1 or 2
- Grade 3 toxicities: injection site pain in 3/46
- Common grade 2 toxicities (2 or more patients)
  - Injection site pain
  - Fever
  - Myalgia
  - Headache
  - Flu-like symptoms

Assays

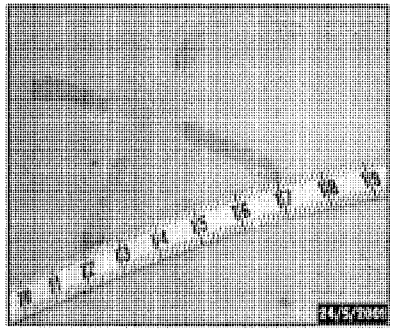
- DTH using NY-ESO-1 protein alone
- Antibody (capture ELISA)
- CD8+ T cells
  - Tetramer: SLLMWITQC
  - Cytospot: gIFN producing CD8+T Cells)
- Assays under development
  - *CD4+ T cells (DC & protein: cytokine secretion)*
  - *Class I epitopes - non HLA-A2*

Antibody titre by cohort

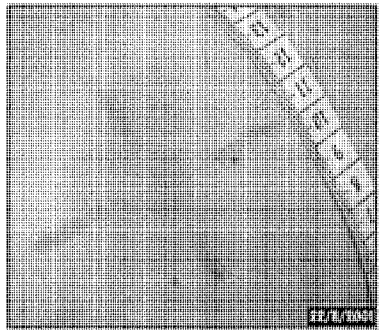


Delayed-type Hypersensitivity: 1 μg protein

Dose C (A2+) : 100μg
NY-ESO-1-ISCOM® /
Placebo
126/KLE

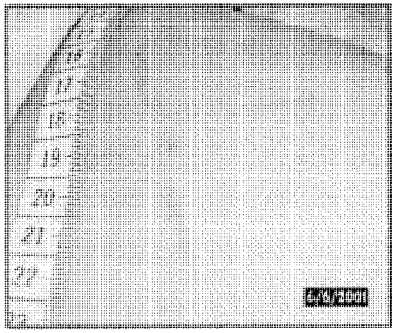


PRE
Erythema = 15
Induration = 3

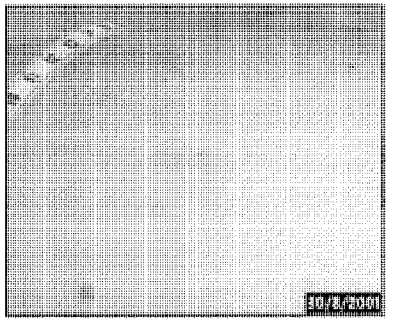


Day 86
Erythema = 60
Induration = 25

Dose D (A2-) : 100μg
NY-ESO-1 Protein /
Placebo
127/JSM



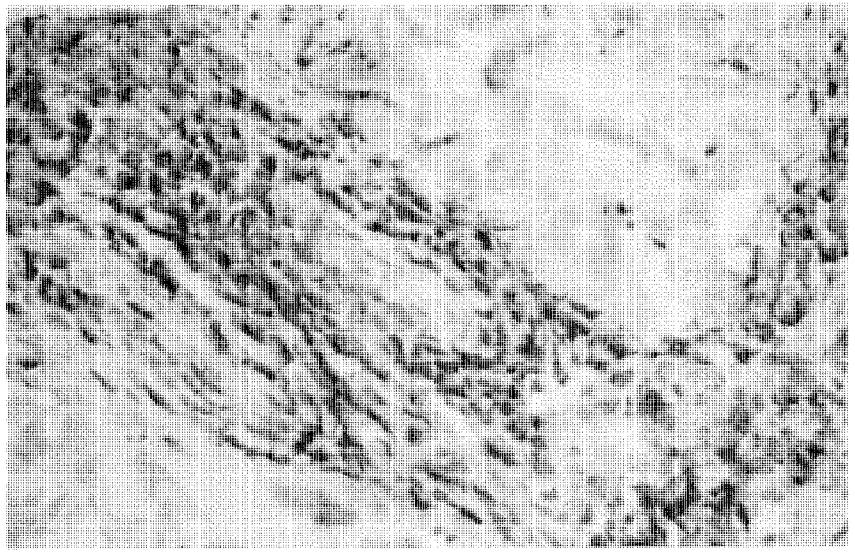
PRE
Erythema = 2
Induration = 0



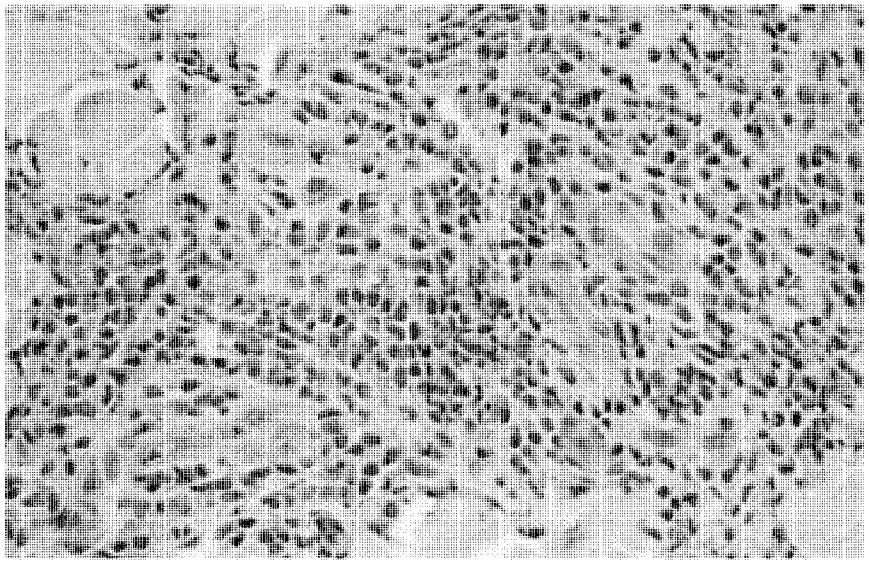
Day 86
Erythema = 12
Induration = 0

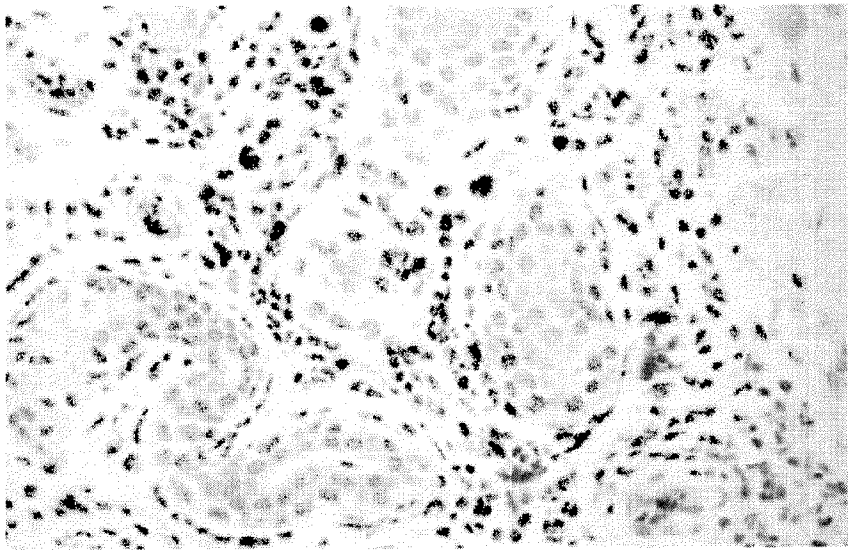
DTH response to 1mg NY-ESO-1 protein

CD4



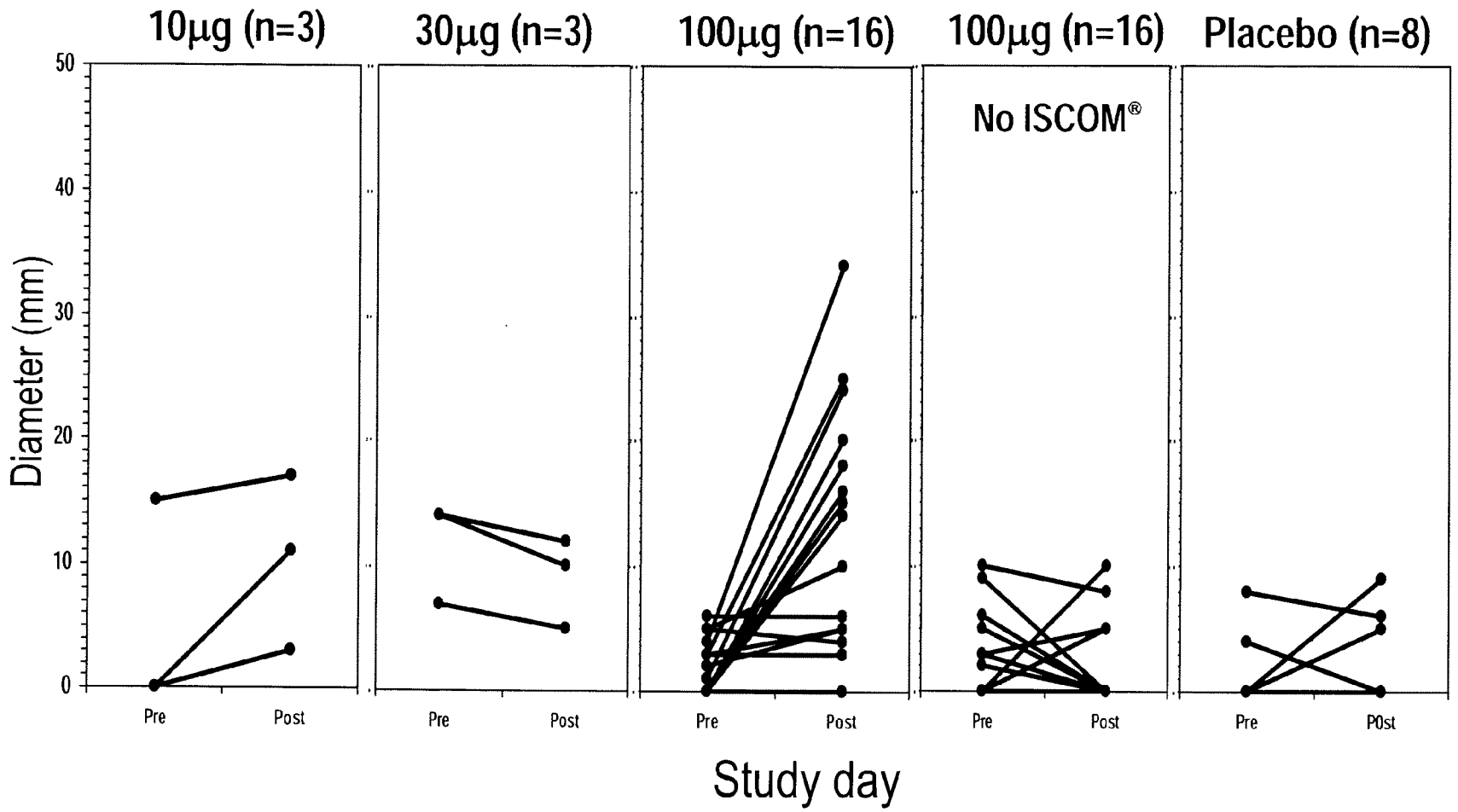
H&E



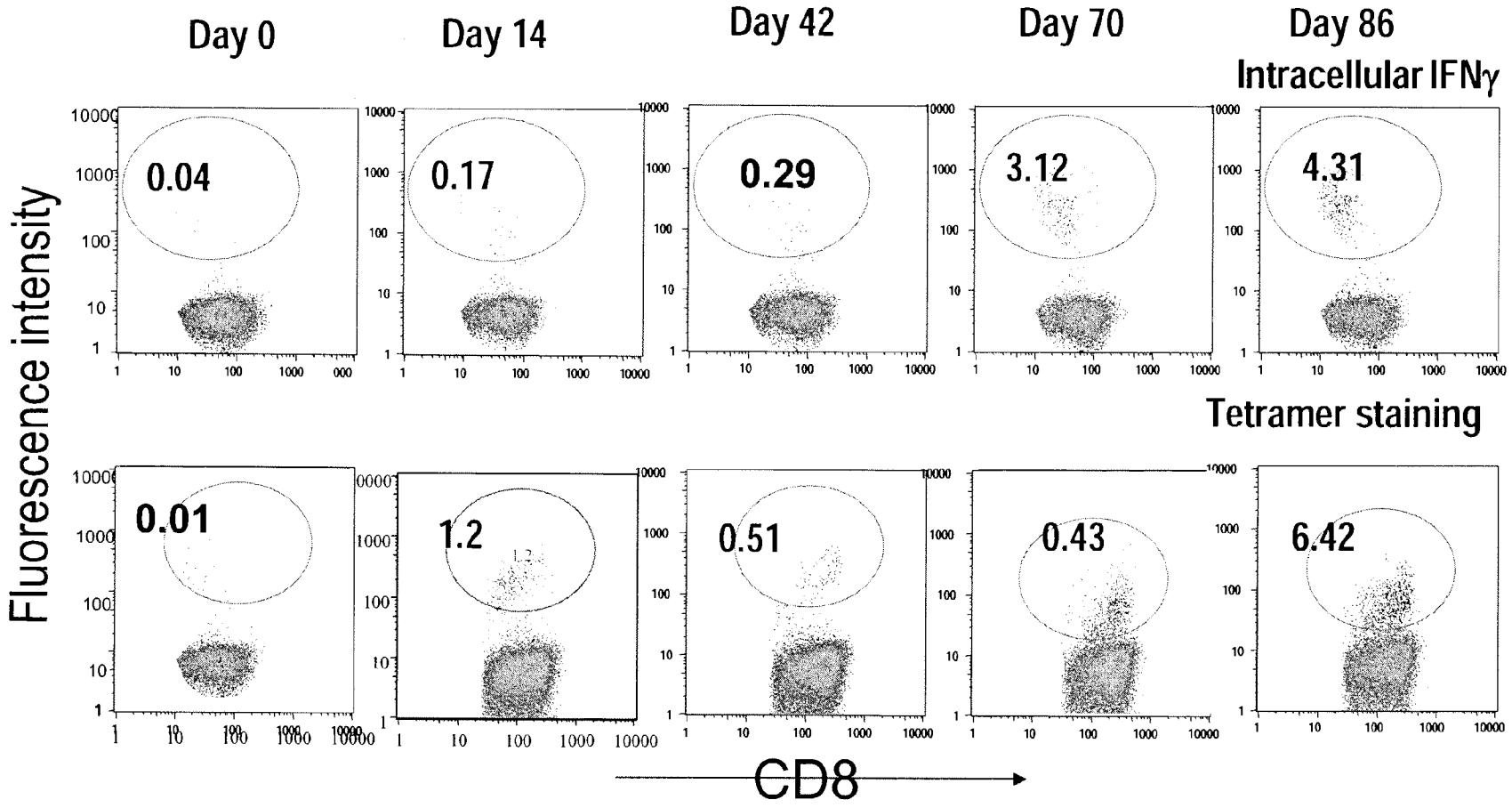


CD8

DTH Induration by cohort



T- cell response: γIFN production
HLA A2+ pt (peptide SLLMWITQC)



Summary Immunological Data

DTH (doubling or greater of induration)

A	B	C	D	Placebo
1/3	0/3	11/16	2/16	2/8

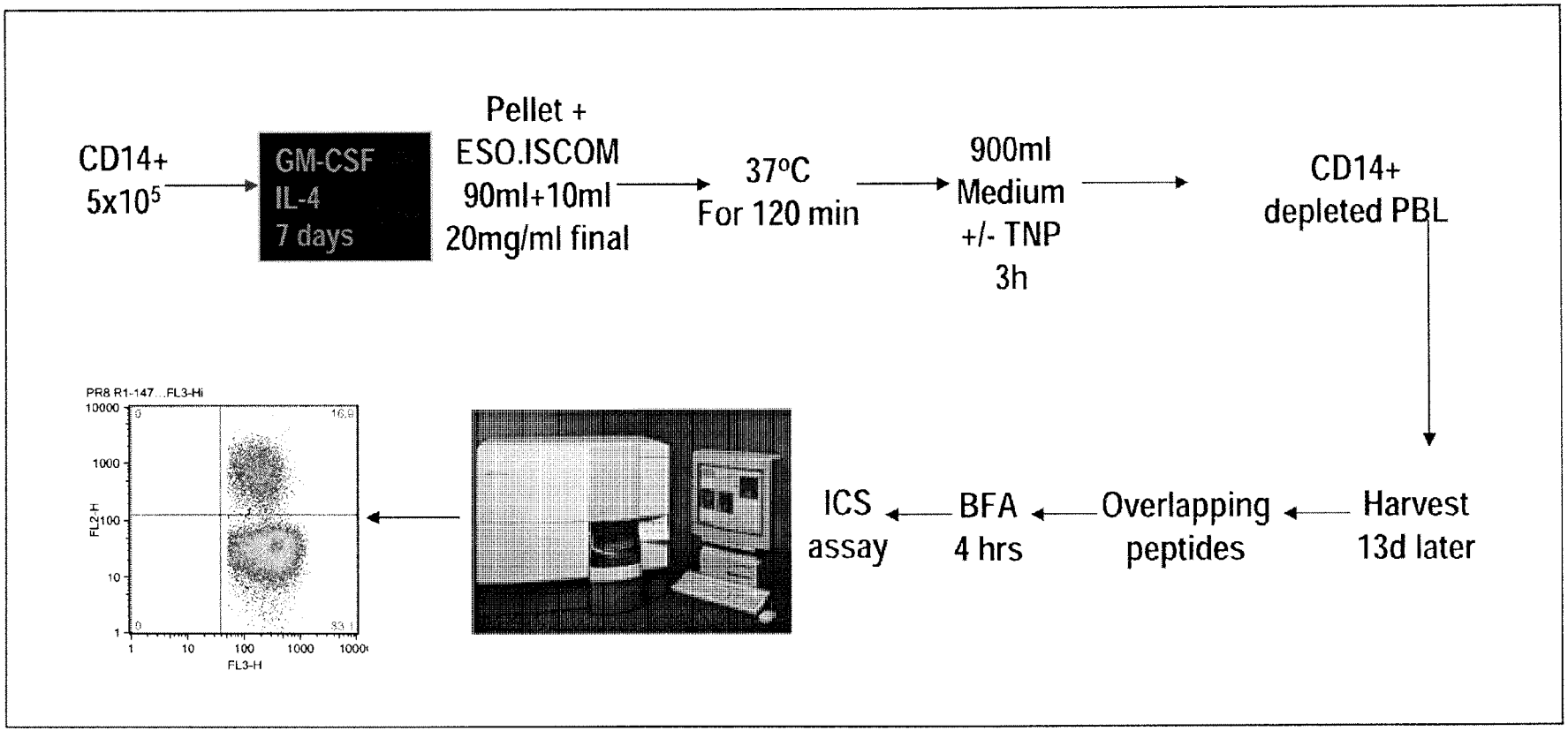
Antibody

A	B	C	D	Placebo
3/3	3/3	16/16	4/16	0/8

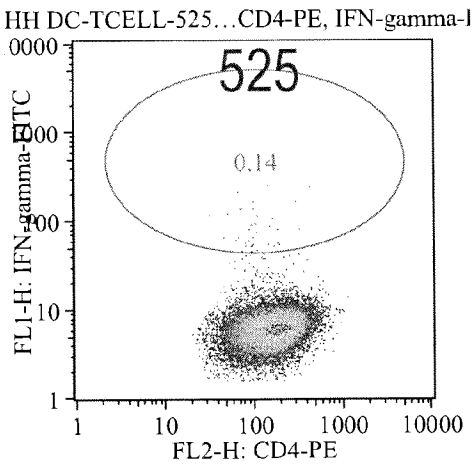
Cytospot & Tetramer

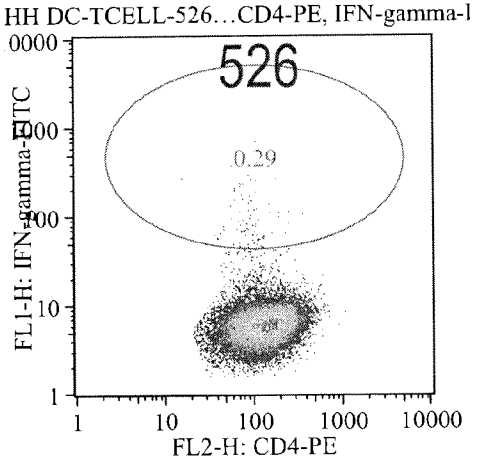
A	B	C	D	Placebo
1/3	0/3	3/8	1/8	0/4

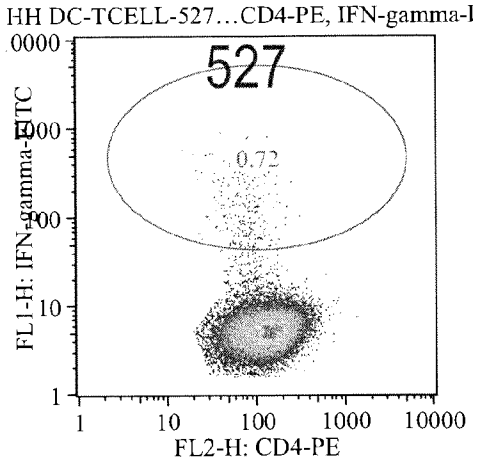
Generation of NY-ESO-1 Specific T cells Using Tumor-Ag-loaded Autologous-DC

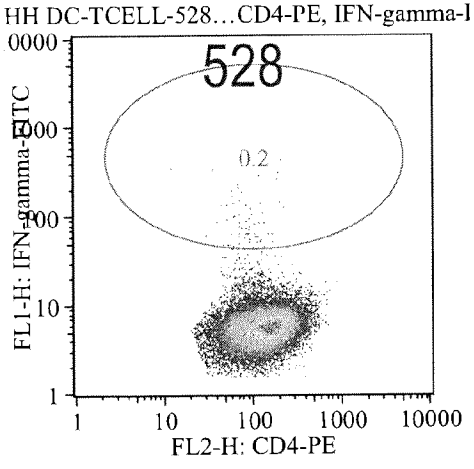


HH T cells generated with DC+ISCOM/NY-ESO-1 and screened with NY-ESO-1 18mer on day 13 after culture

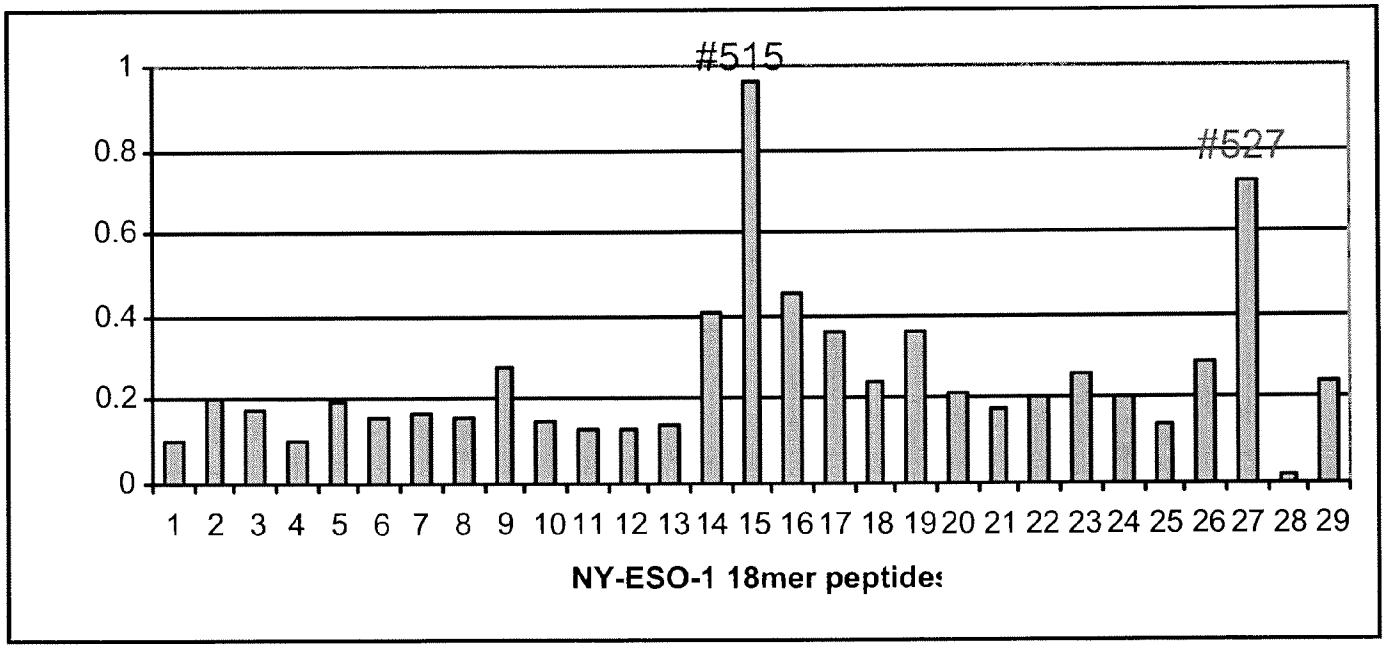








T cells generated with DC+ISCOM/NY-ESO-1 and screened with 18mer peptides at day 13 after culture

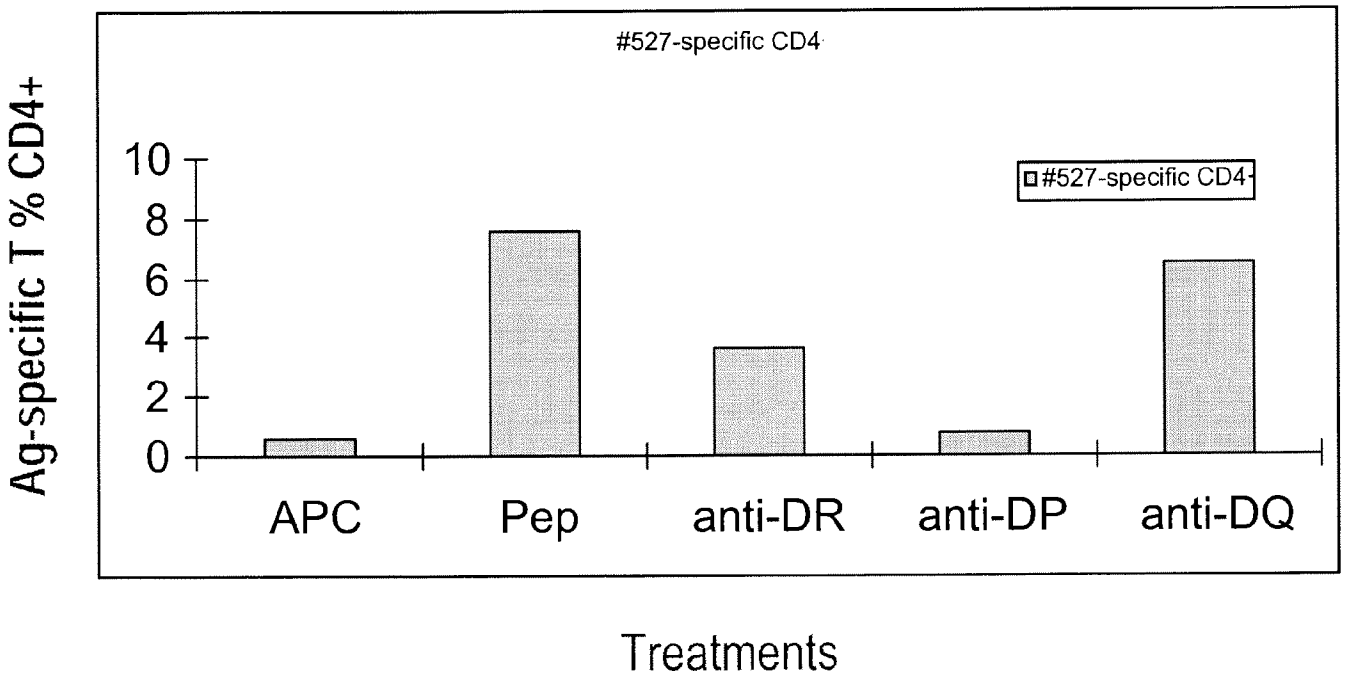


Further characterisation of DC generated CD4 T cells

- Lines & clones established
- Antibodies
  - Anti DR, DP, DQ
- LCL lines
  - LCL auto: DR1, DR2, DP4
  - LCL 9080: DR1, ---, ---
  - LCL 9014: ---, DR2, ---
  - LCL T291: ---, DR2, DP4
  - LCL T282: ---, ---, DP4
- Tumor lines
  - NW38: DR1, ---, ---, NY-ESO-1(+)
  - LAR1a: ---, DR2, ---, NY-ESO-1(+)
  - SK-Mel 37: ---, ---, ---, NY-ESO-1(+)

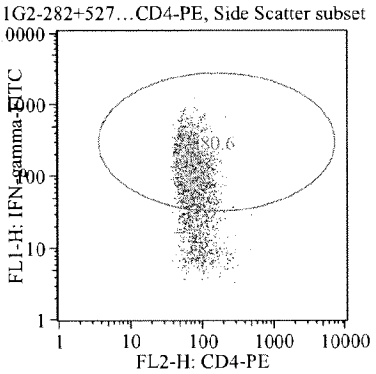
#527-specific CD4+ T cells are DP restricted

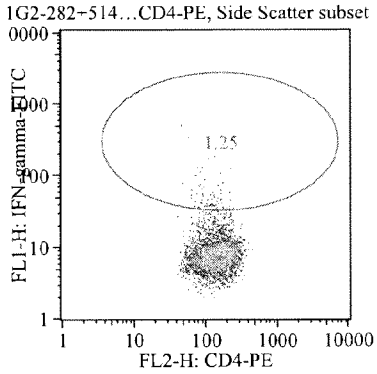
(DC stimulated then #527-pulsed BCL stimulated 2x)



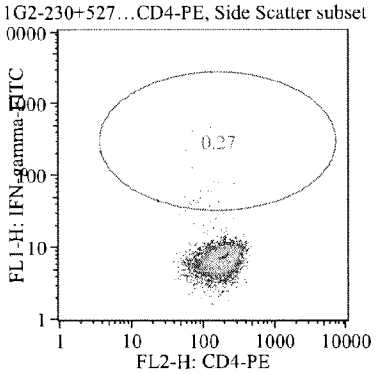
#527-specific T cells are DP4 restricted

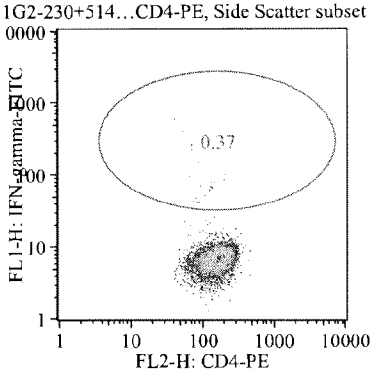
DP4+ LCL (282)





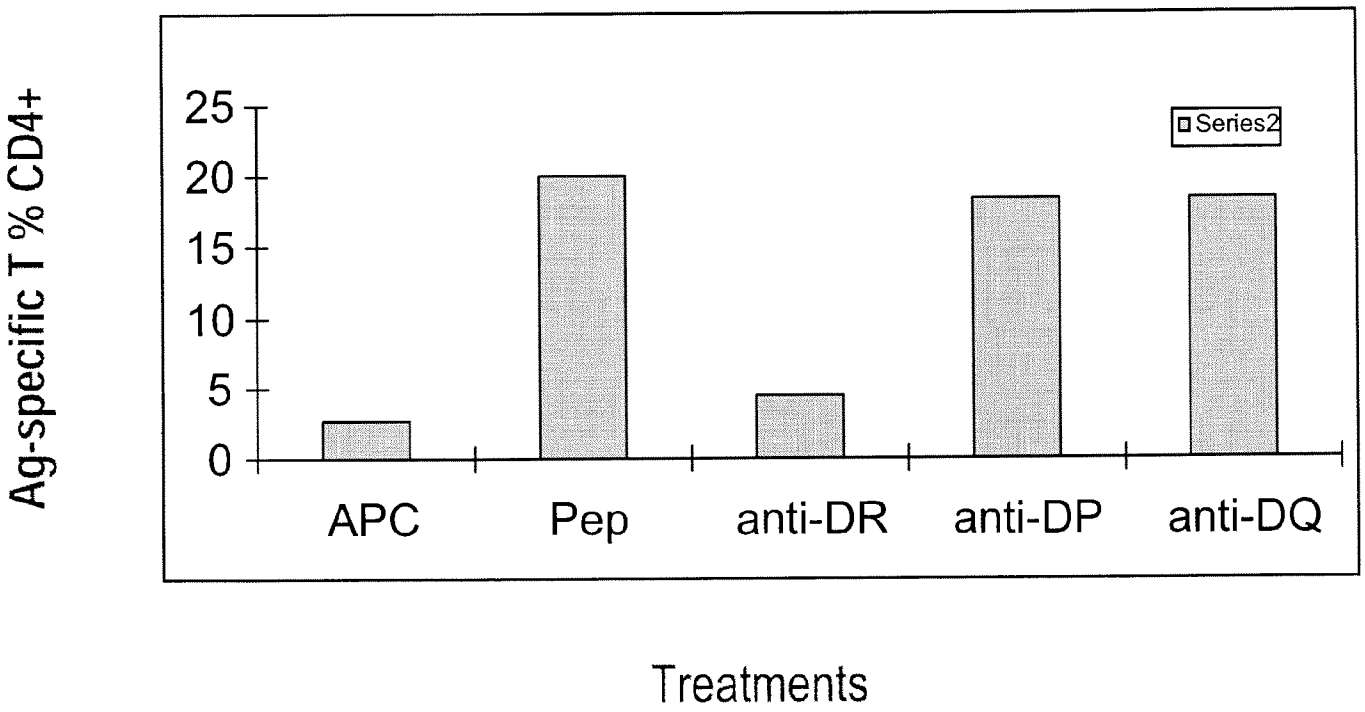
DP4- LCL (230)



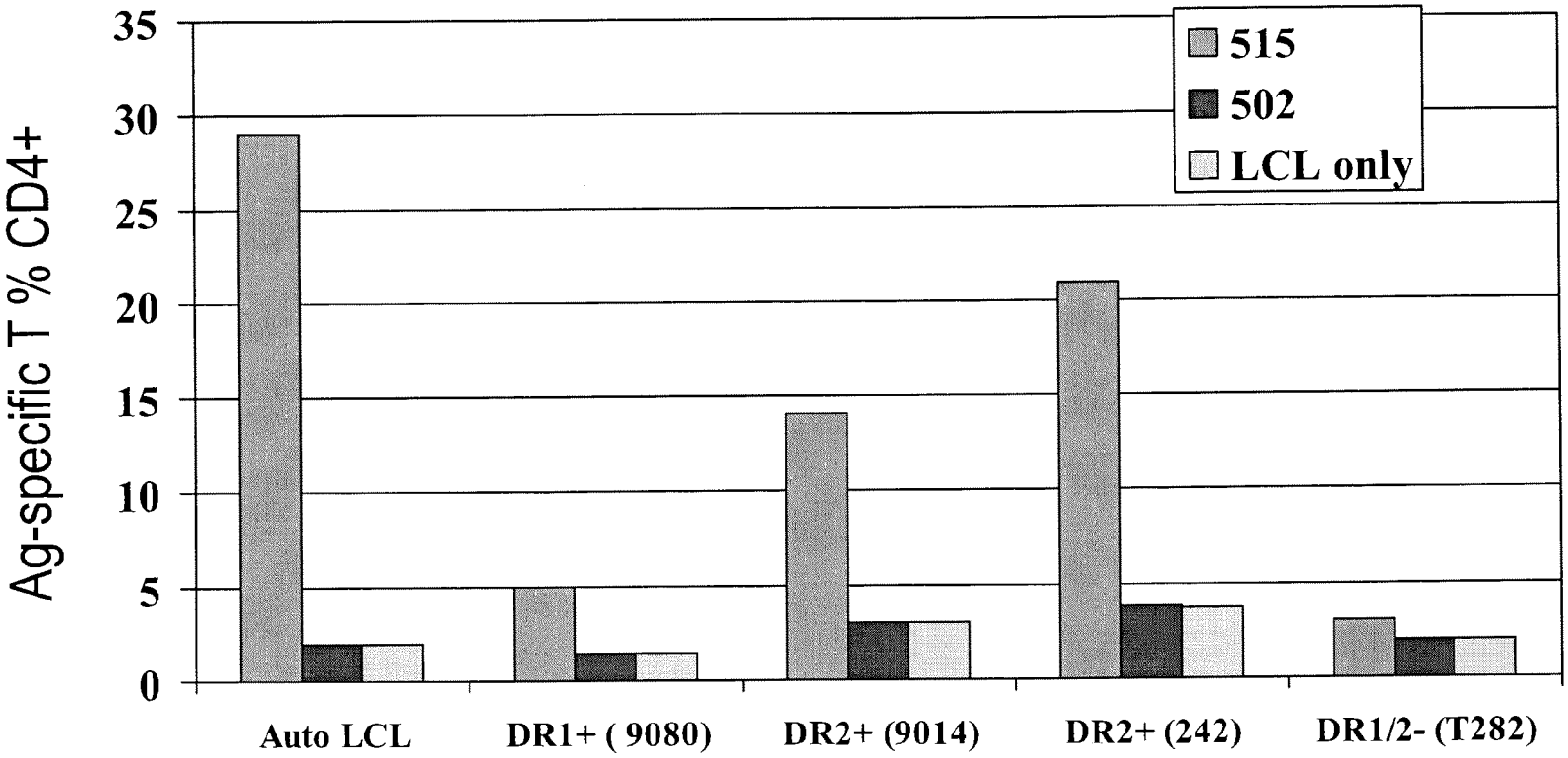


#515-specific CD4+ T cells are DR restricted

(DC stimulated then #515-pulsed BCL stimulated 2x)

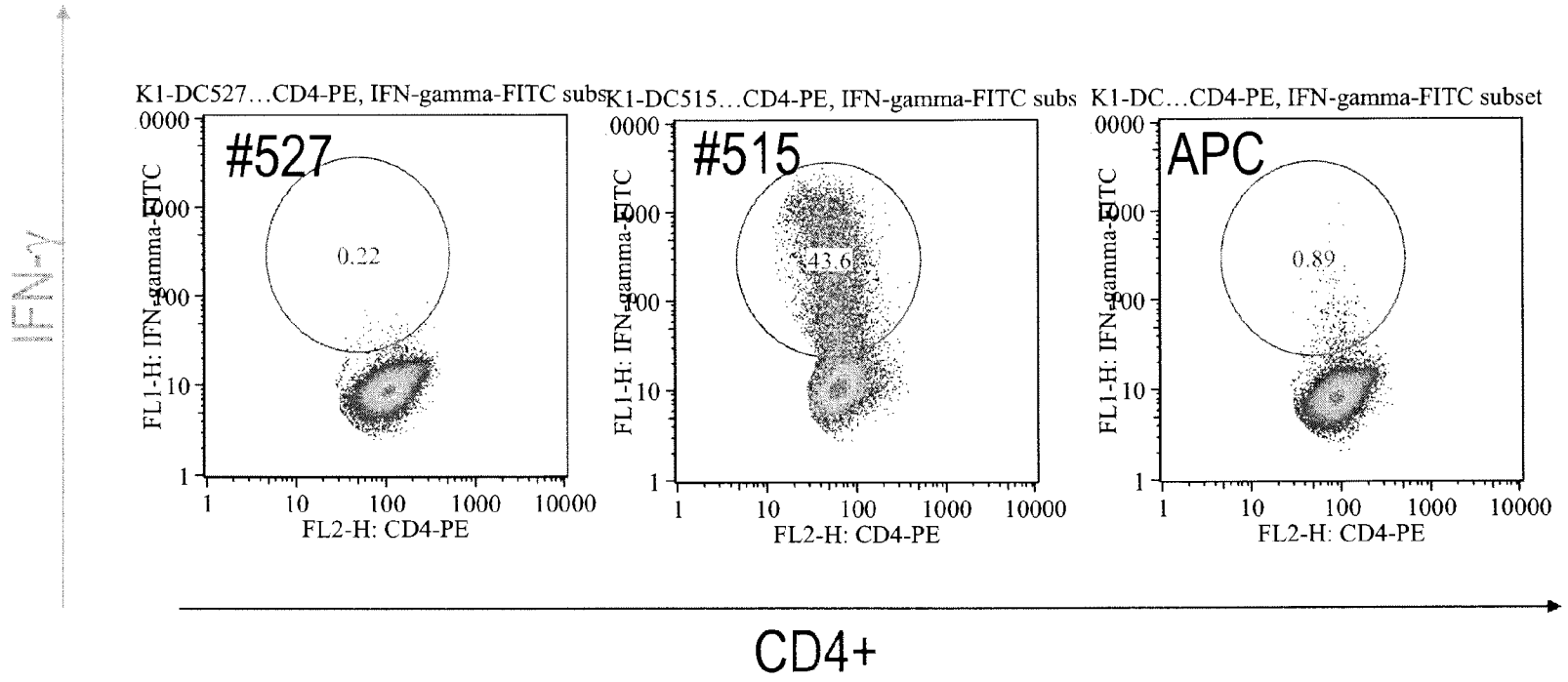


DR restriction of #515-specific T cells
(#515 2xstimulations)

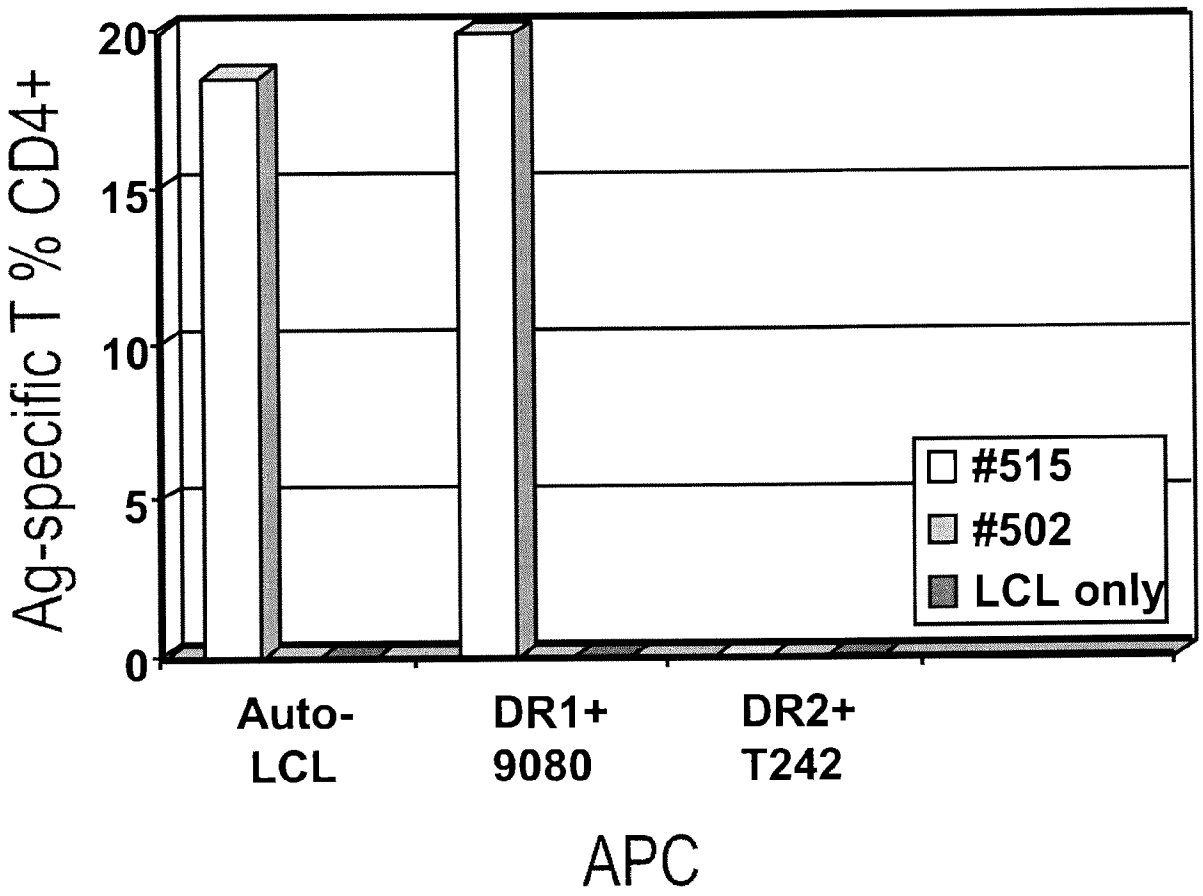


#515-specific T clone K1

generated with #515-pulsed DC



T clone K1 is DR1-restricted



Conclusions

- NY-ESO-1 ISCOMs vaccinations were safely tolerated
- NY-ESO-1 ISCOMs generated both humoral & cellular responses
- ISCOM adjuvant generated superior DTH and antibody responses
- Cytospot assay in HLA A2+ve patients: positive in 1 level A pt (with prior Ab response), 3/8 level C patients and 1/8 level D patients.
- These responses were seen in patients with and without pre-existing antibody titres
- There was a good correlation between tetramer & cytospot data
- There is evidence of CD4 responses to 2 novel epitopes in first level C patient tested - this analysis is ongoing

Acknowledgements

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Lisa Pugliese

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Brian Tait

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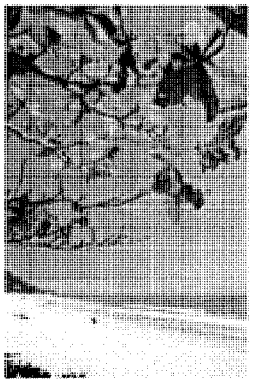
Cancer Vaccination

NY-ESO-1 as a model antigen



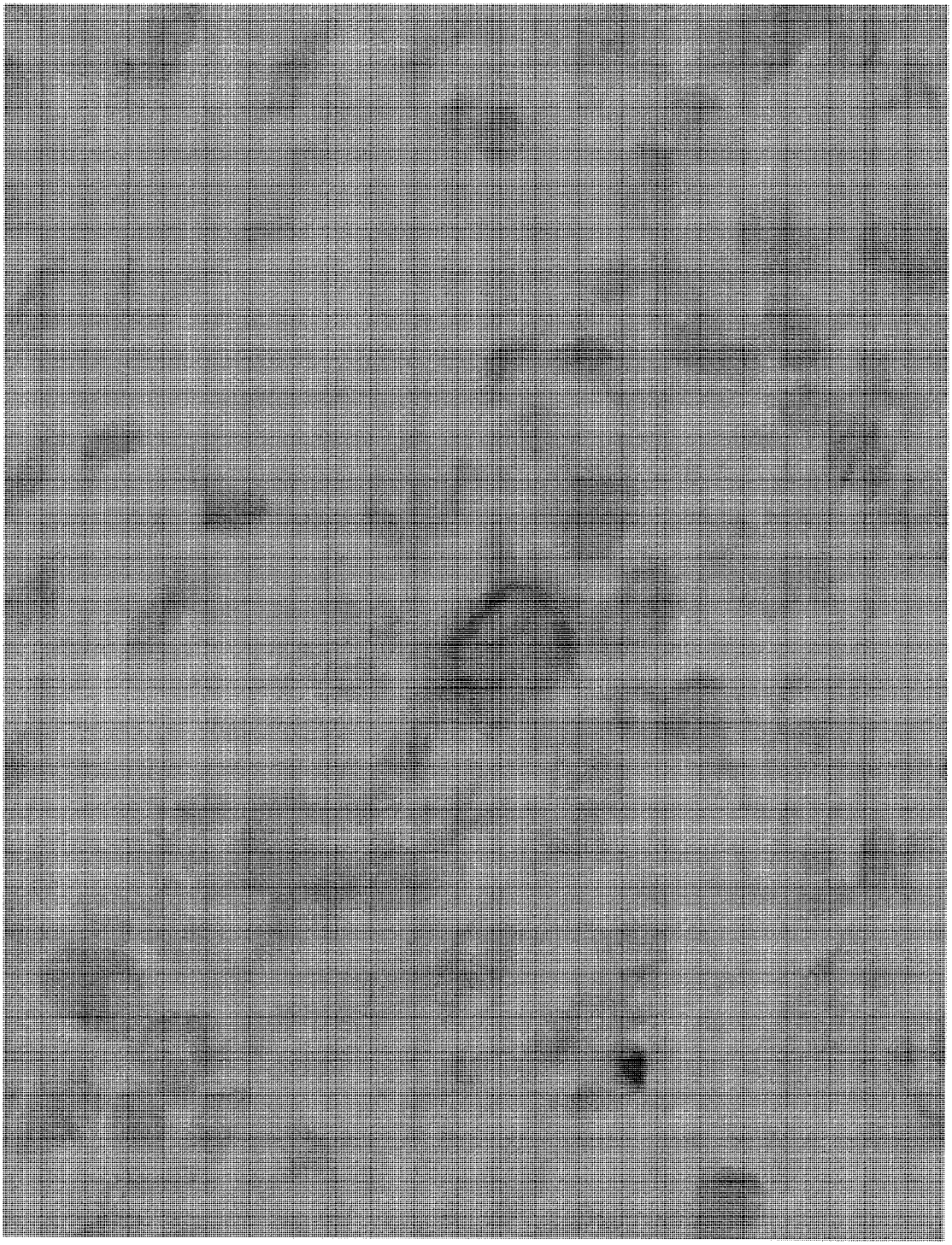
- Human cancers & immunity
- Melanoma
- NY-ESO-1 the antigen
- Vaccination with NY-ESO-1
- Clinical directions

Cancer Risk following Renal Transplant

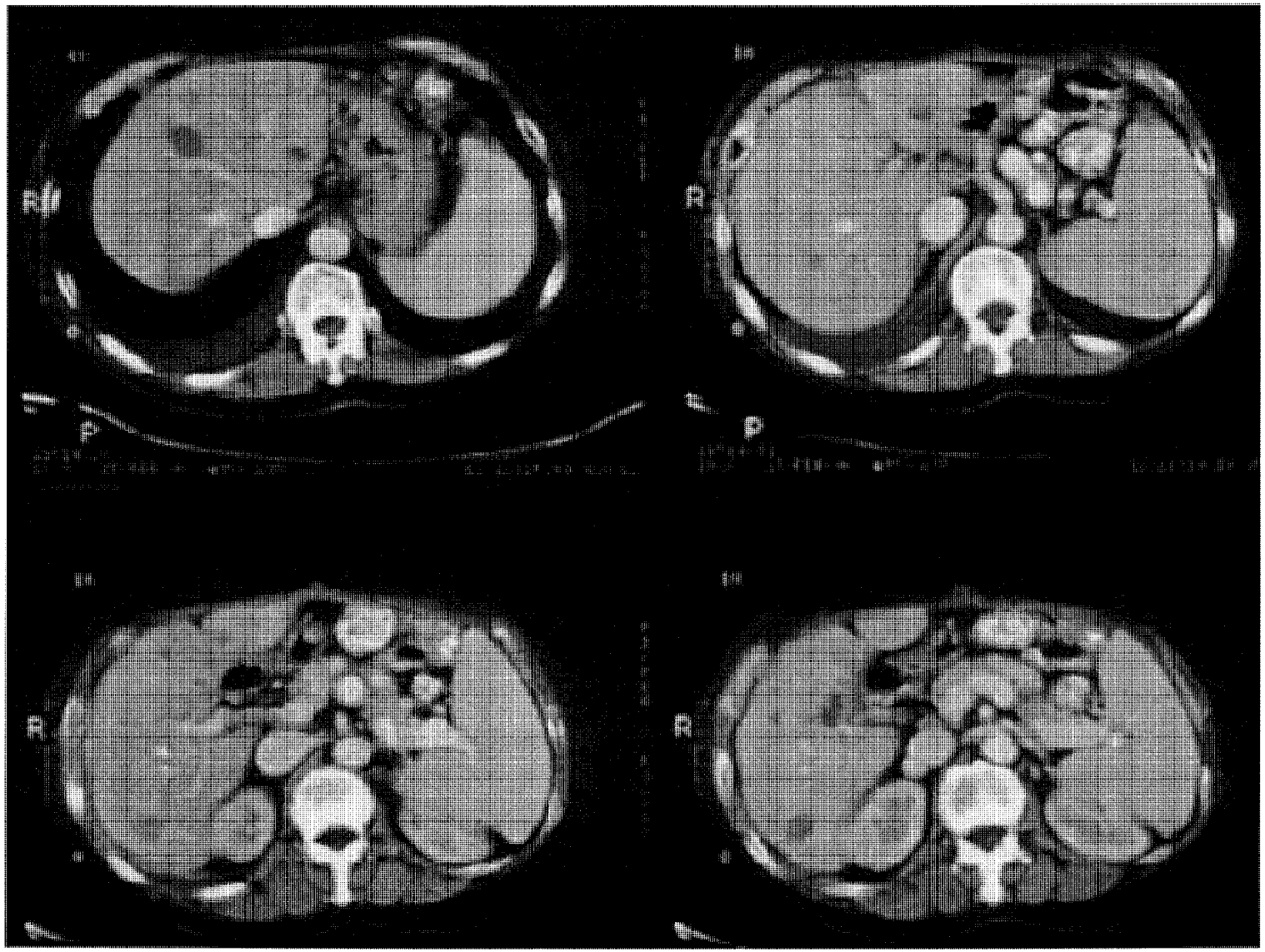
ANZ Dialysis & Transplant register 1997 8618 patients

Cancer	Number	Risk Ratio
CNS Lymphoma	17	>1000
Ureter	10	250
Parathyroid	2	200
Kaposi Sarcoma	18	86
Vulva/Vagina	40	43
Penis	7	24
Cervix	65	17
Bladder	54	7
Kidney	8	7
NH Lymphoma	83	7
Liver	8	6
Colon	50	2
Breast	42	1

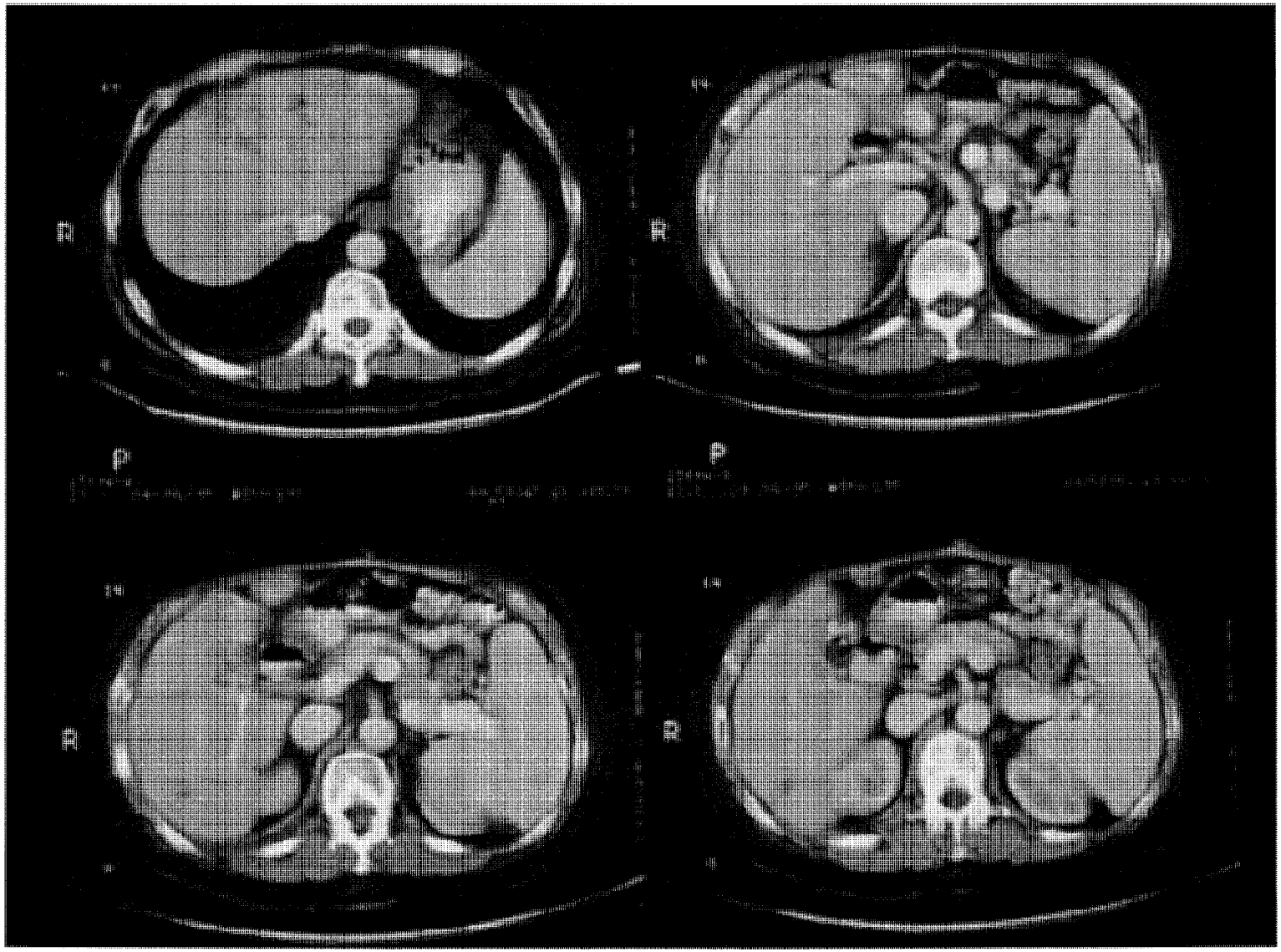
Lymphoma in a Liver Transplant recipient



Post CHOP x3

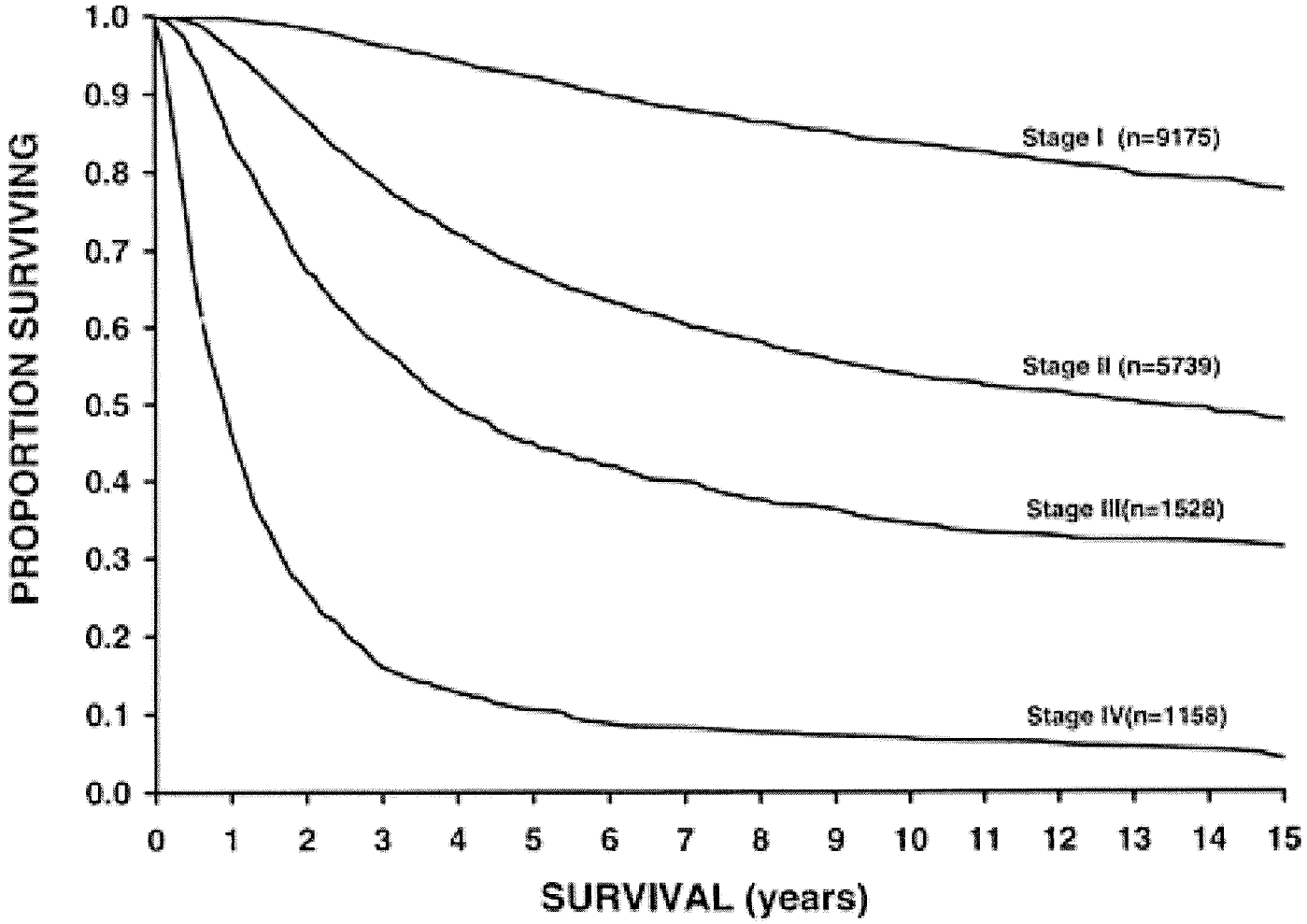


Off Treatment



Spontaneous immune responses can occur in some cancer patients

Melanoma Stage & Survival



Balch et al JCO Vol 19 2001: p 3635

Malignant Melanoma

- Spontaneous regression observed
- Lymphocyte infiltration of primary tumor is associated with better prognosis
- Responds to immune manipulation
  - non - specific :IFN-α, IL-2
  - Vaccines
- Autoimmune phenomena
  - Vitiligo
  - chorioretinitis
- Antibody responses have been documented eg to gangliosides, NY-ESO-1

Melanoma associated chorioretinitis

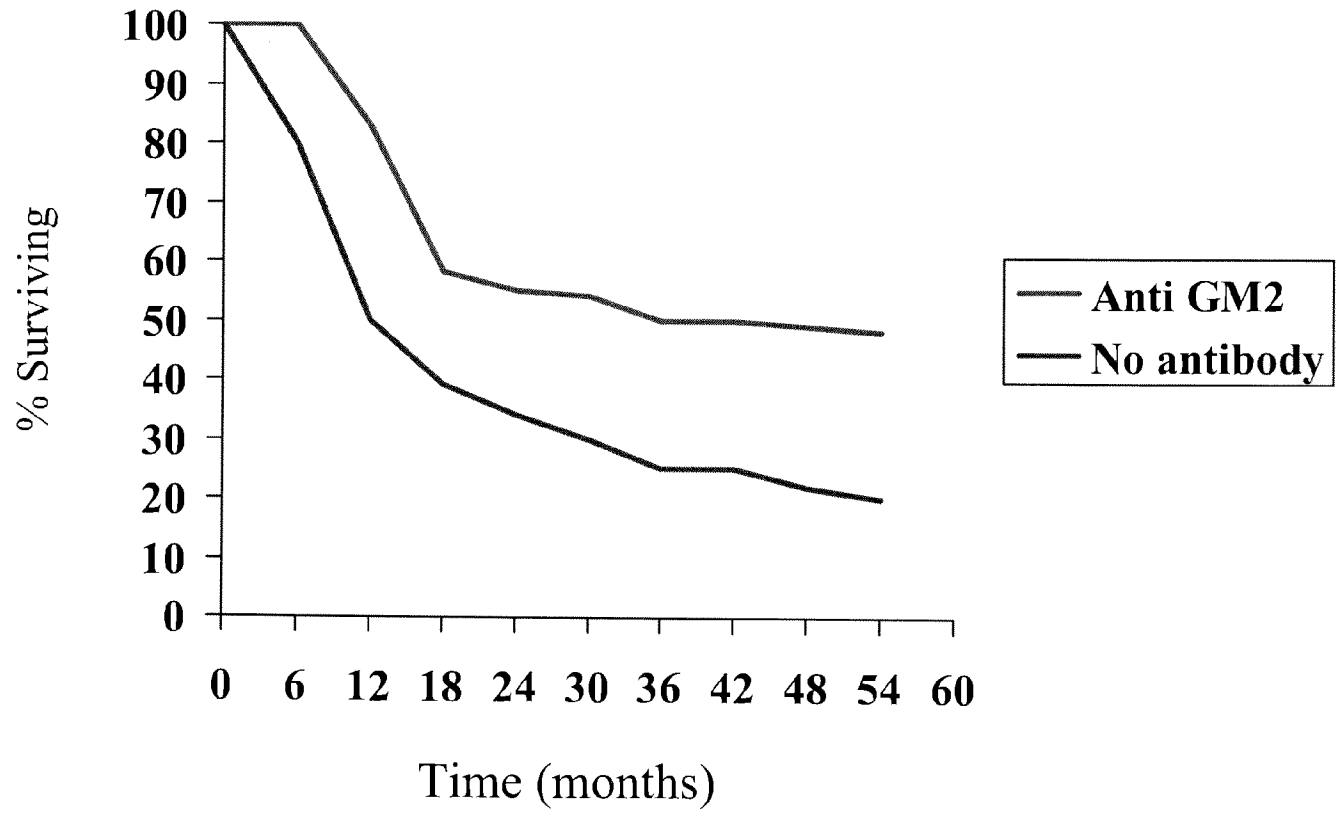
- Level 5 melanoma diagnosed ‘86
- recurrence lung & lymph nodes excised Sep 90
- multiple cutaneous recurrences
- Chemotherapy
- Night blindness, reduced central vision Aug/91
- Serum: immunofluorescence on unfixed retina (bipolar cells)
- Melanoma - associated chorioretinitis
- Small bowel metastases resected Apr/94
- Currently free of disease

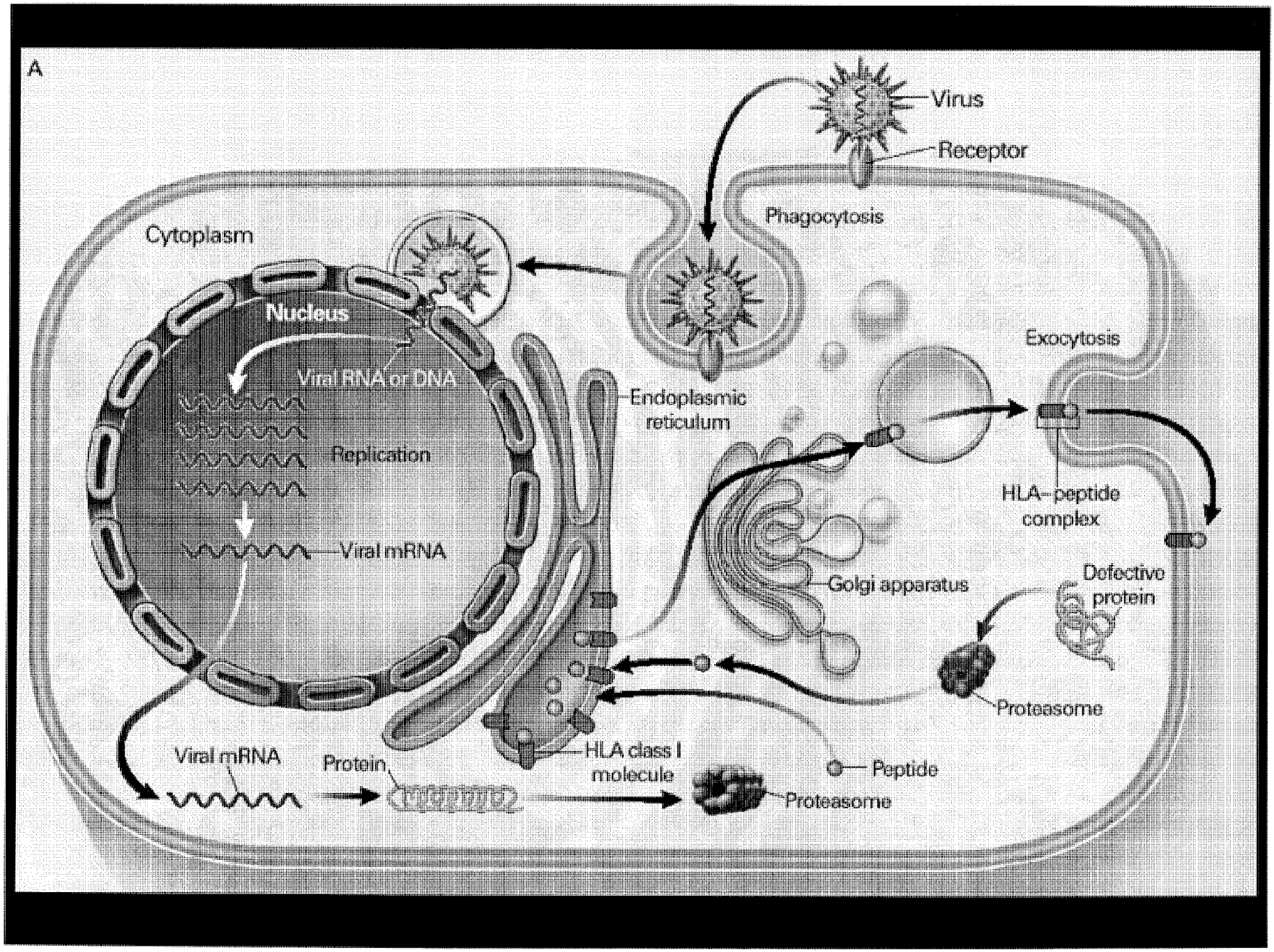
Antigens identified:

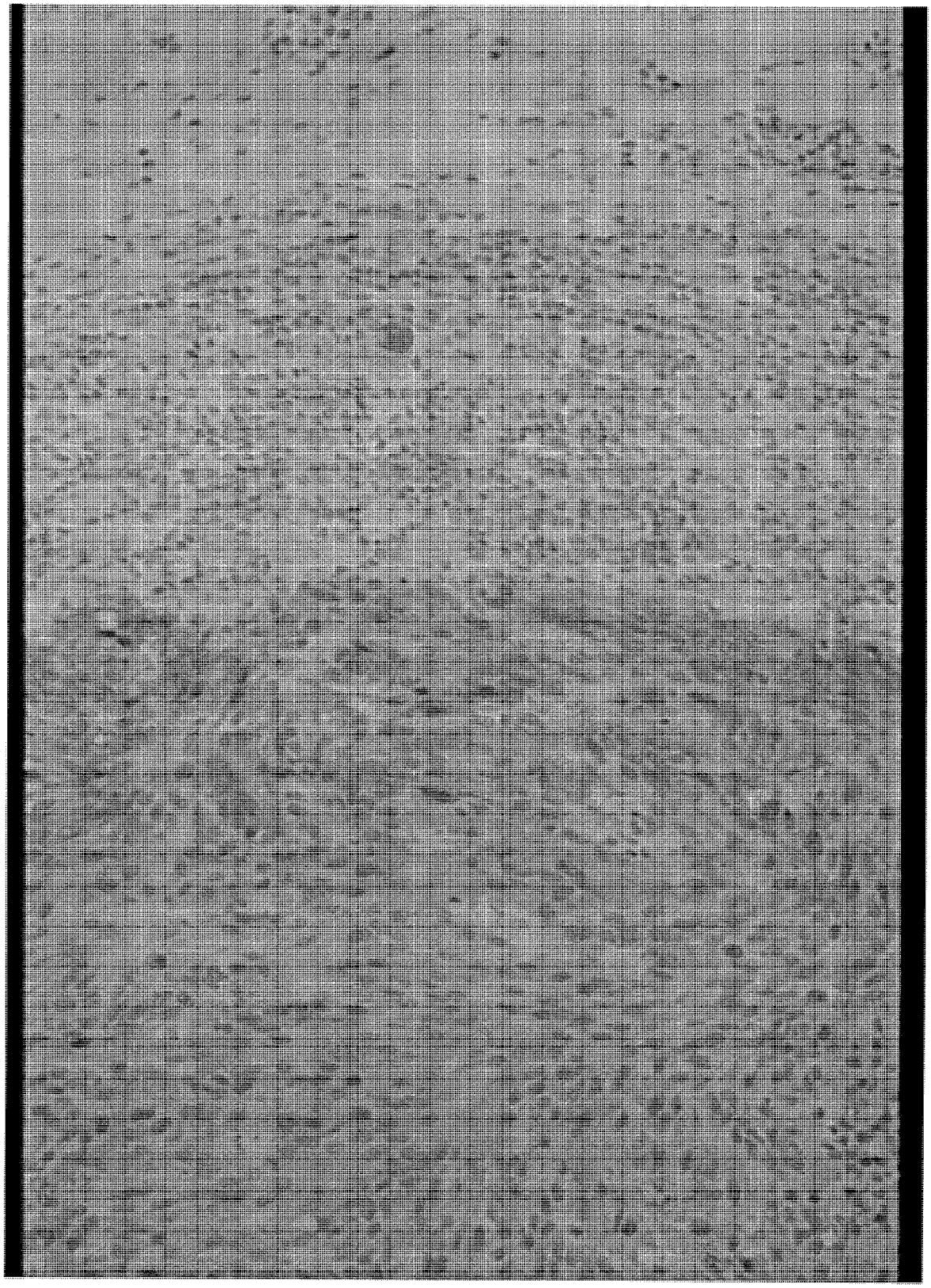
- Cytotoxic lymphocyte targets:
  - Differentiation Ags
    - MelanA/MART-1
    - gp100
    - Tyrosinase
  - CT antigens
    - MAGE family
    - NY-ESO-1
- Antibodies
  - Gangliosides GM2
  - Spontaneous : observed in ~10%
  - associated with improved survival

Improved survival in stage III melanoma patients with GM2 antibodies: a randomized trial of adjuvant vaccination with GM2 ganglioside. Livingston PO, et al

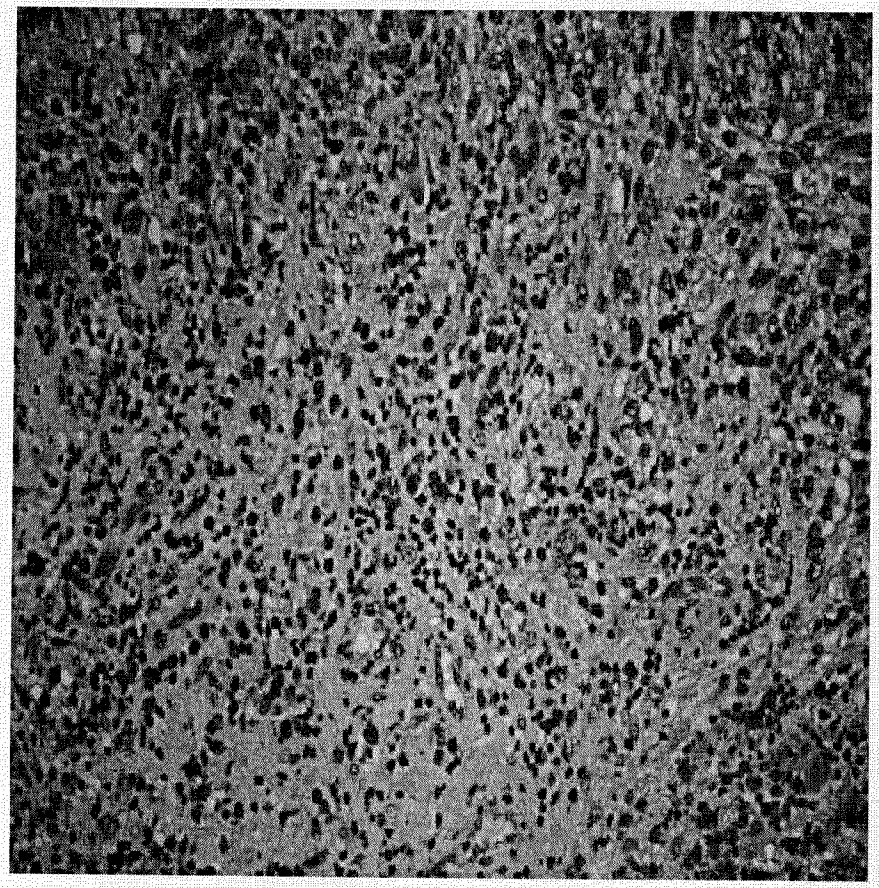
J Clin Oncol 1994 May;12(5):1036-1044

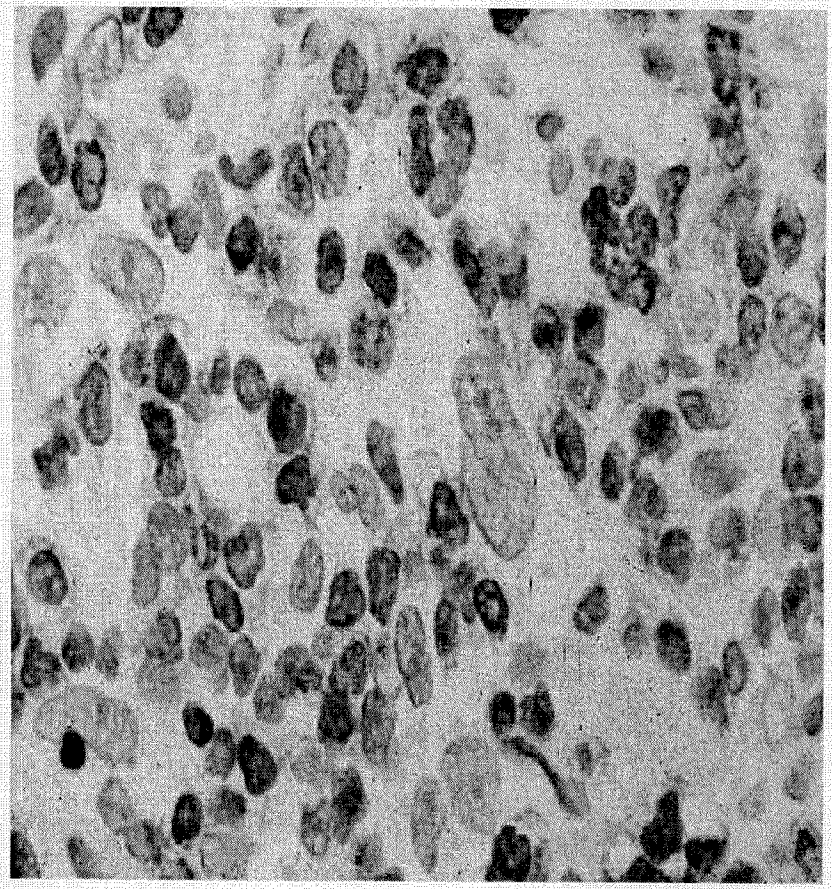



A
Virus
Receptor
Phagocytosis
Cytoplasm
Nucleus
Viral RNA or DNA
Replication
Viral mRNA
Endoplasmic reticulum
Exocytosis
HLA–peptide complex
Golgi apparatus
Defective protein
Proteasome
Viral mRNA
Protein
HLA class I molecule
Peptide
Proteasome



Immune responses to tumour antigens may affect clinical outcomes in some patients



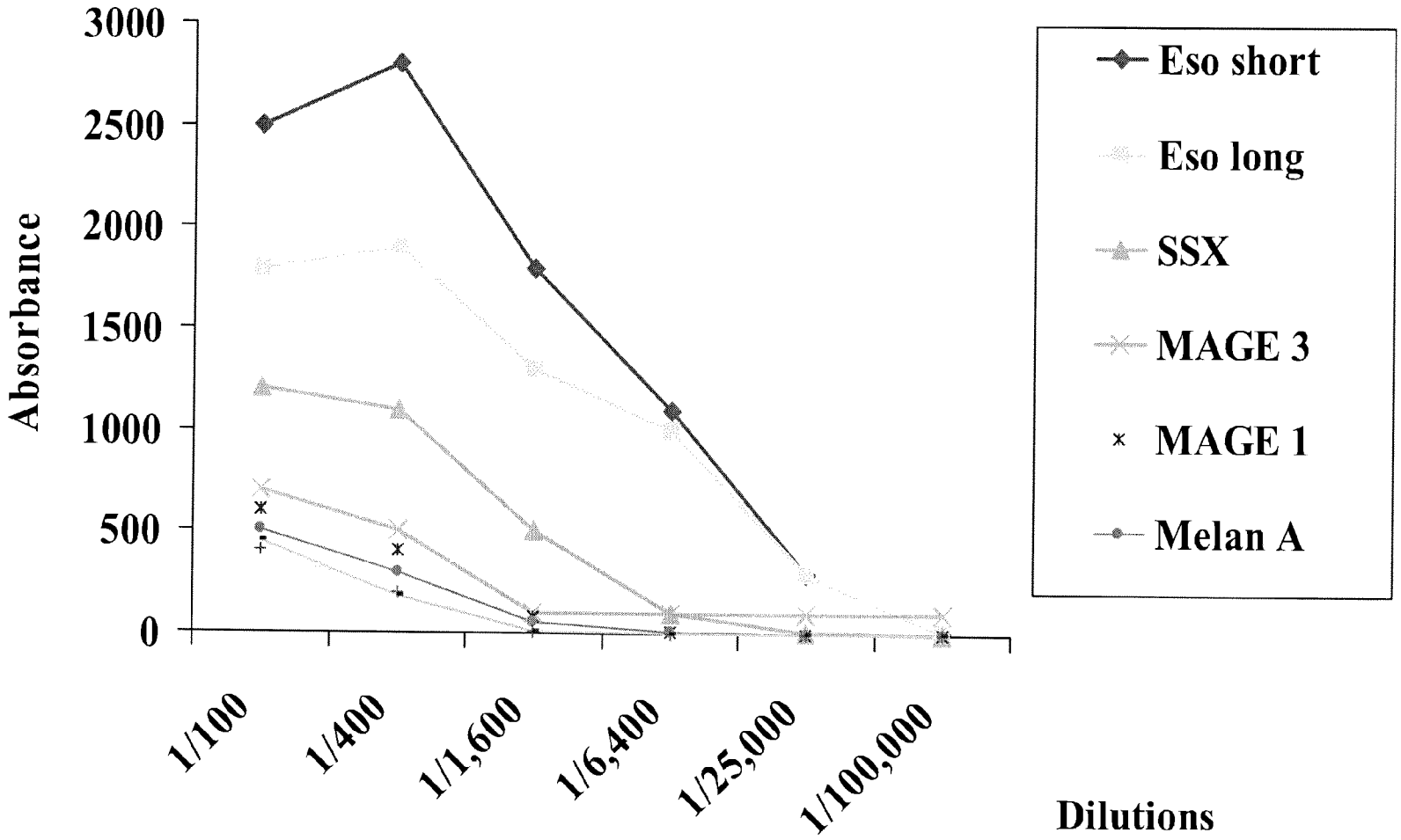


Melanoma Infiltrates with CD8+ Lymphocytes

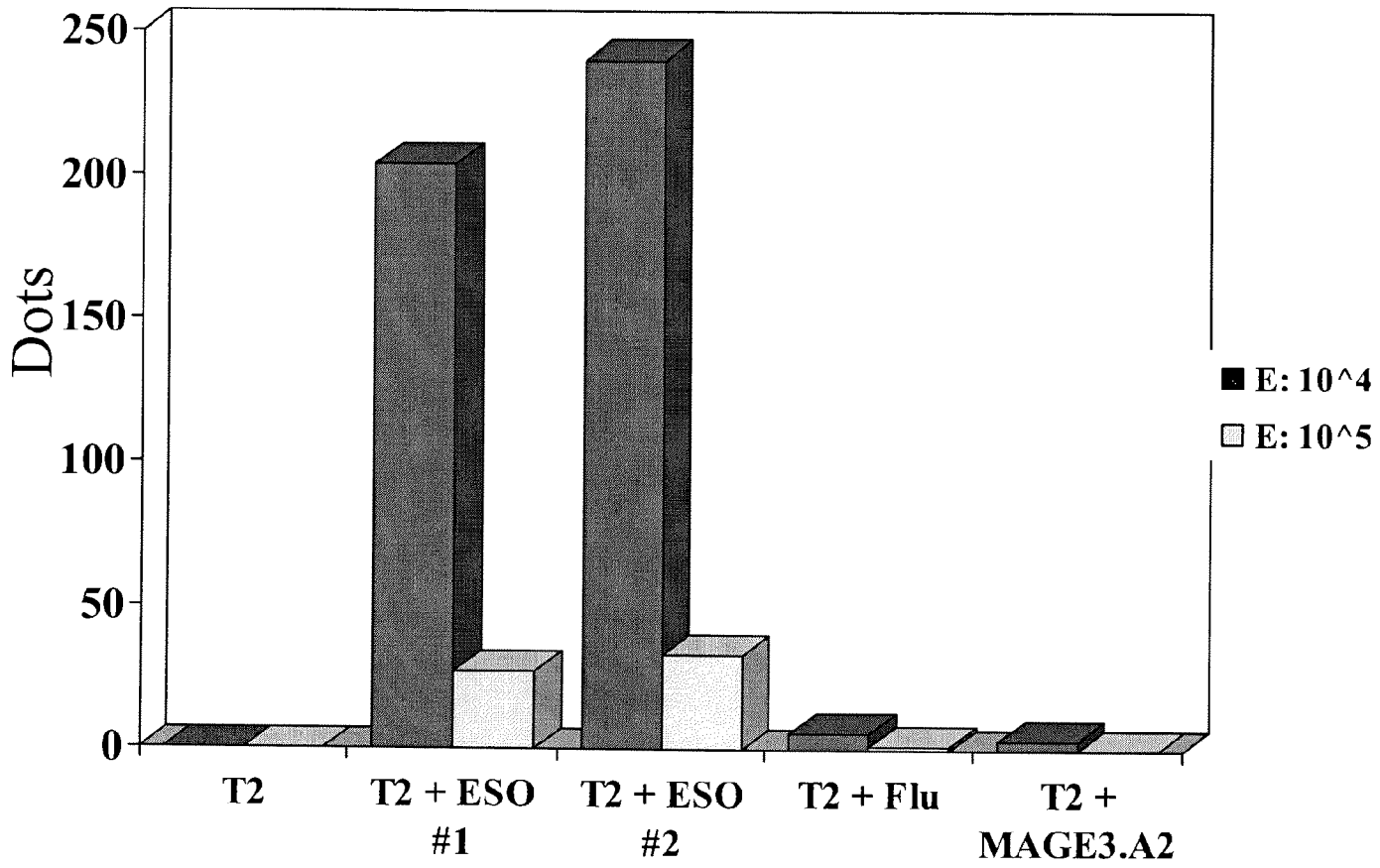
Indolent Melanoma

- Initial diagnosis: Dec 87
- Adjuvant BCG
- Relapse in iliac and retroperitoneal lymph nodes: Nov 93
- Vitiligo
- slowly progressive - treated with chemotherapy and radiotherapy
- Extensive necrosis of tumor on CT scan
- plasma call infiltrate in tumor
- Died Apr 97

Indolent Melanoma: ELISA for Tumor Ags



ELIspot assay: NY-ESO-1



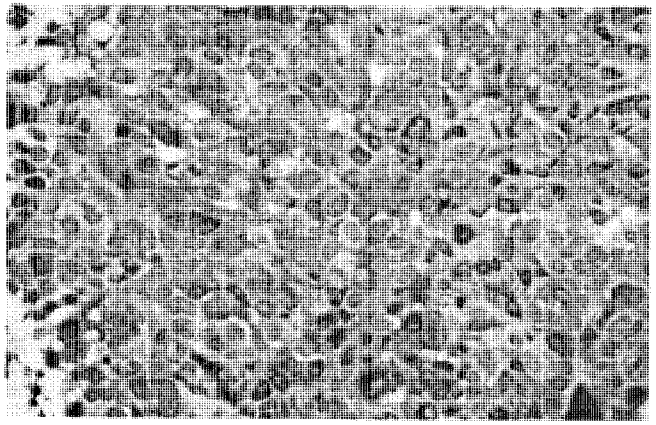
NY-ESO-1

CT 'Cancer testis' antigen
Cytoplasmic
Unknown function
180 amino acids
Expressed in testis, trophoblast
Variety of cancers
- Melanoma
- Hepatocellular Carcinoma
- Lung
- Bladder
- H&N
- Synovial sarcoma
- Breast

Highly immunogenic
Epitopes restricted by Class I
- HLA A2
- HLA Cw3, Cw6

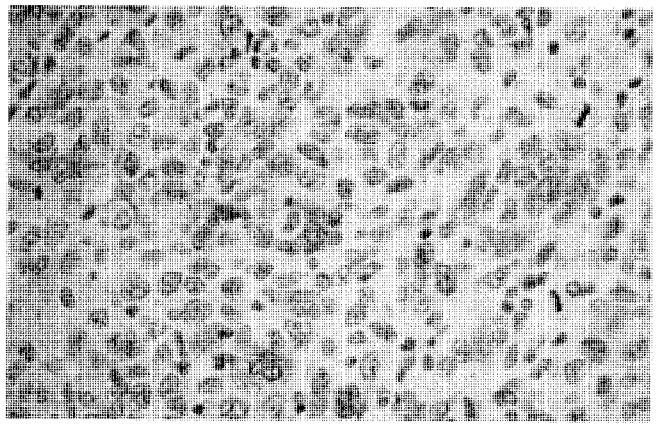
Class II
- HLA DP4
- DR53
- DR4

Heterogenous expression
- RT-PCR
- Antibody (ES121, E978)



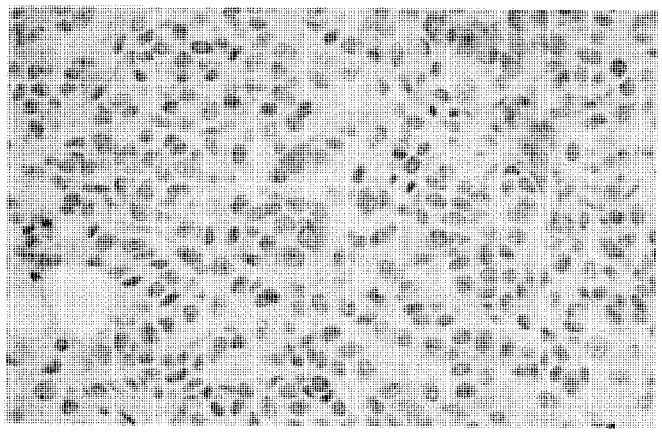
NY-ESO-1 Rich

IHC >50% cells staining, ++ or greater
20/120 melanomas **(18%)**
PCR +ve: 20/20 (100%)



NY-ESO-1 Intermediate

20/120 Melanomas **(18%)**
PCR +ve: 15/20 (75%)



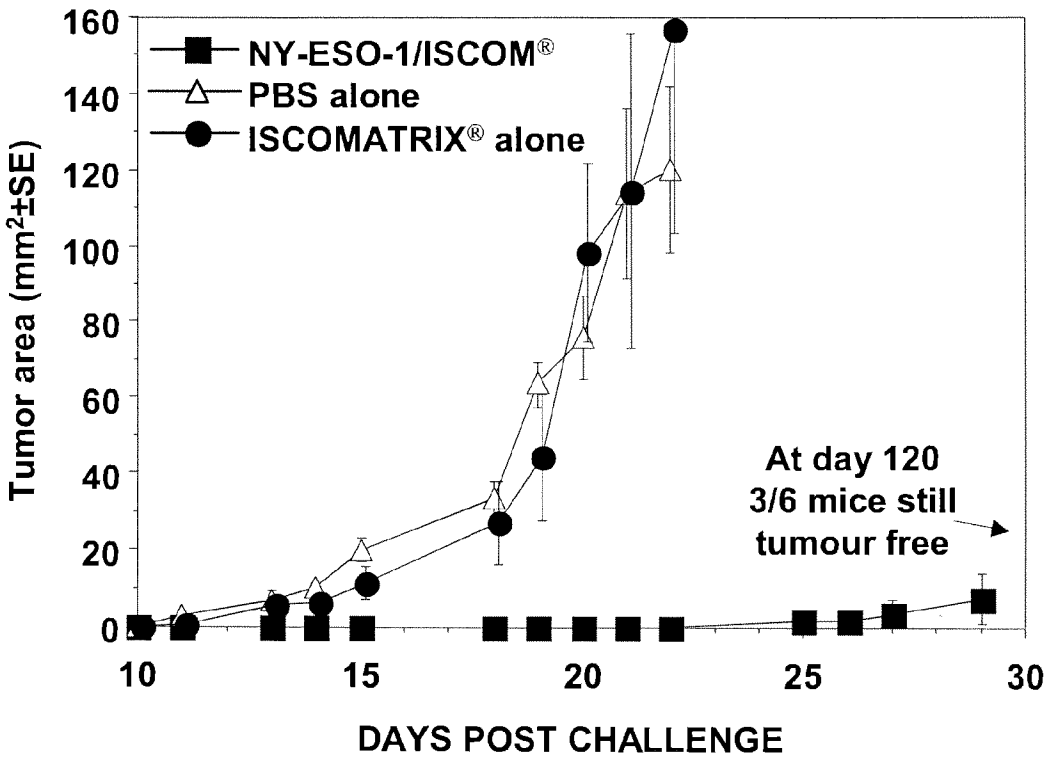
NY-ESO-1 Poor

<25% + or <5% any intensity
6/120 melanomas **(5%)**
PCR +ve: 3/6 (50%)

NY-ESO-1 Negative

66/120 melanomas **(59%)**
PCR +ve: 14/66 (21%)

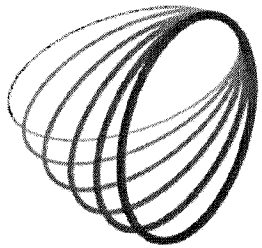
Figure 8. Maraskovsky et al.,



LUDWIG INSTITUTE FOR CANCER RESEARCH

CSL LIMITED

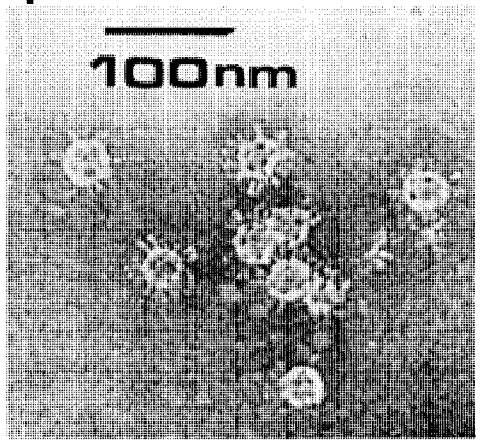
A phase I study of NY-ESO-1 ISCOM® in patients with NY-ESO-1 positive cancers and minimal residual disease



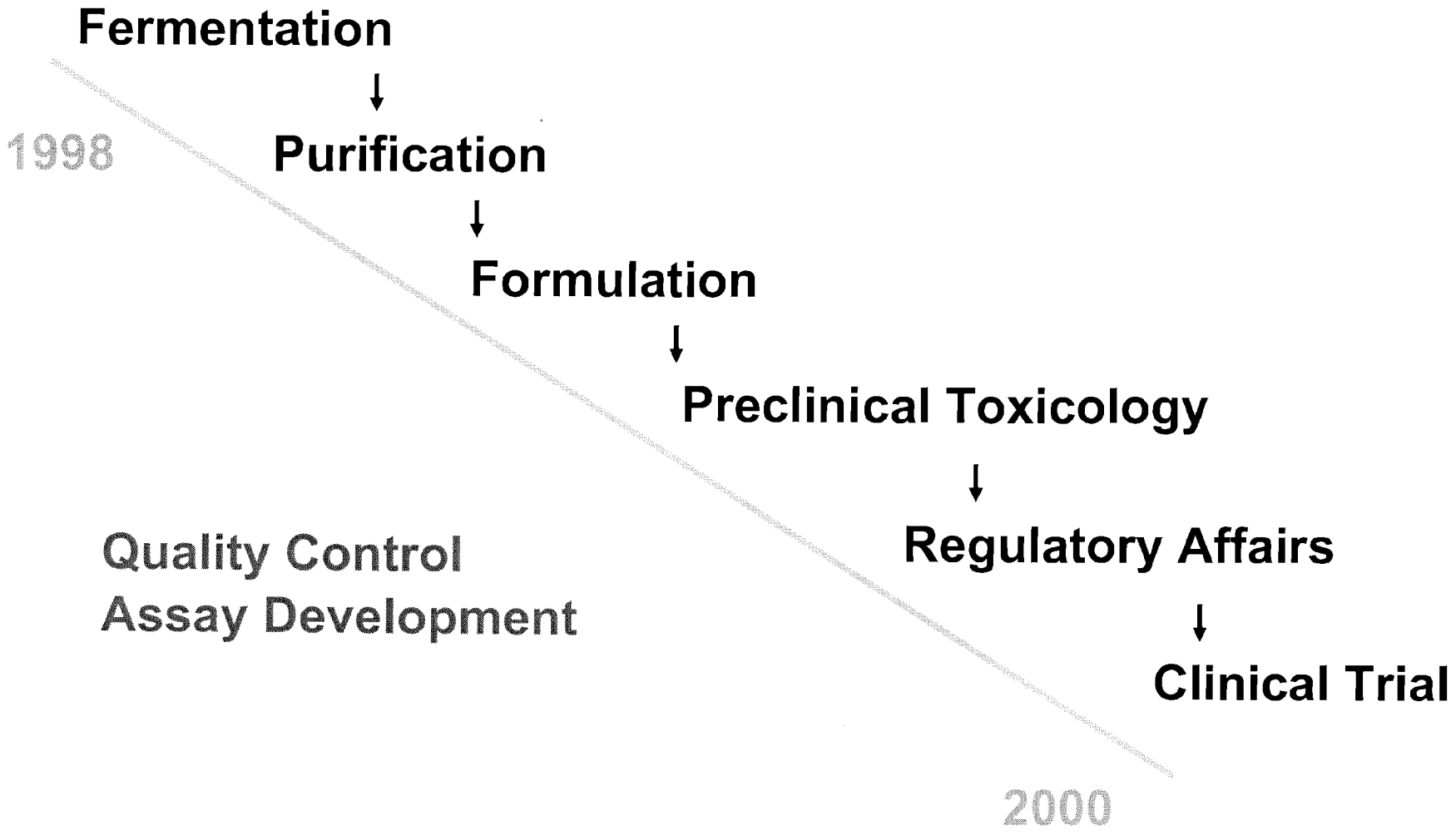
CDCT

ISCOM®

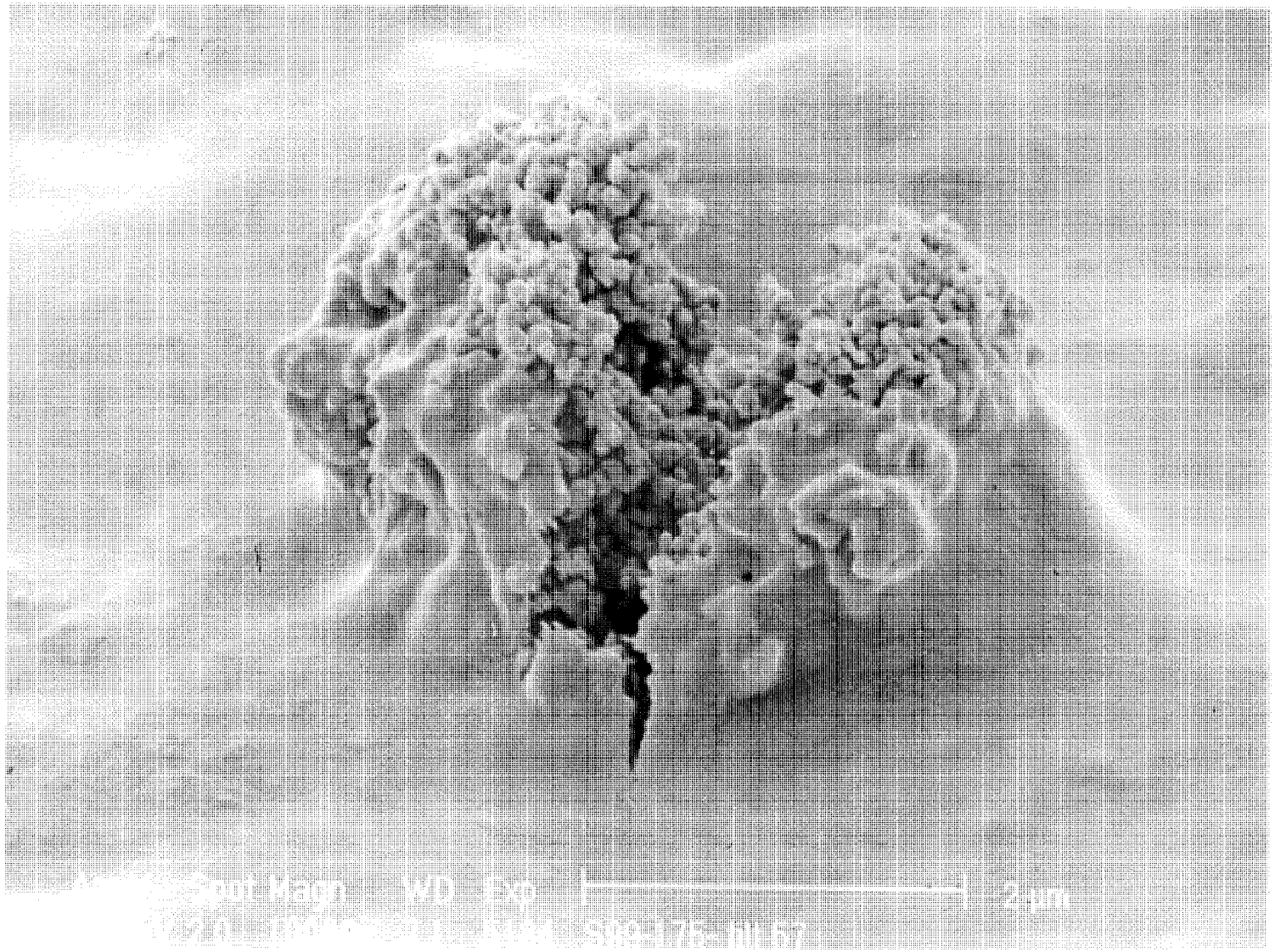
- Adjuvants
  - humoral → Aluminium salts
  - cellular → ?
- Immuno Stimulating COMplexes
  - ISCOM™
  - ISCOMATRIX™



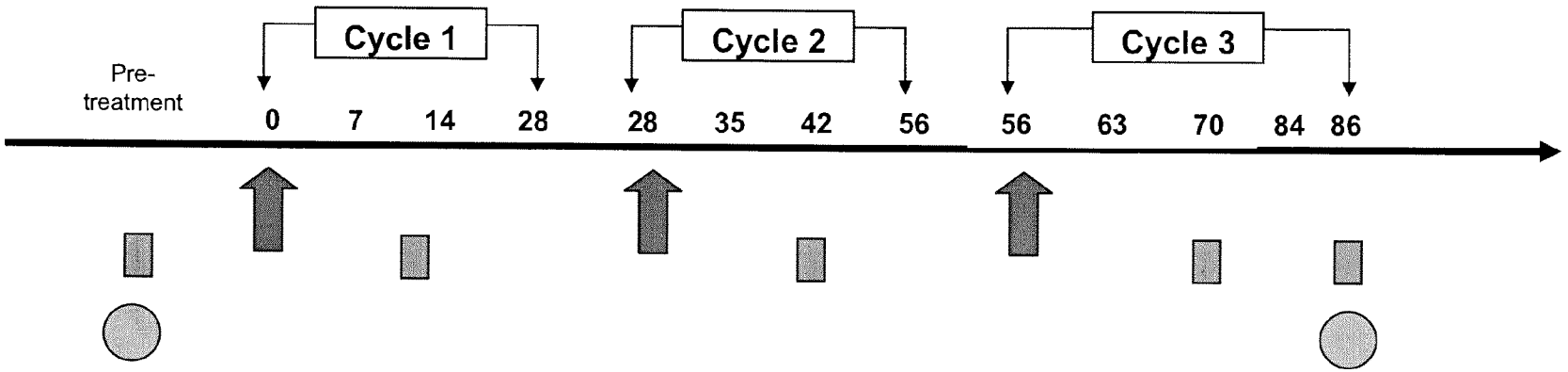
Vaccine Production Timeline



Scanning EM of NY-ESO-1 ISCOM®



Study Design



Vaccination

DTH

T-cell assays & serology

Patients

- Total 46
- 3 parts
  - 1 NY-ESO-1/ISCOM®
    - 3 pts/cohort
    - Dose levels A 10ug & B 30ug
    - Only HLA A2+ patients for purposes of immunological assays
  - 2 NY-ESO-1/ISCOM® - dose level C
    - Dose 100ug expanded to 20 patients
    - 10 HLA A2+ve (2 placebo), 10 HLA A2-ve (2 placebo)
  - 3 Protein alone - dose level D
    - 100ug expanded to 20 patients
    - 10 HLA A2+ve (2 placebo), 10 HLA A2-ve (2 placebo)

Cancer Types

On Study	**51**
Melanoma*	**46**
Ca Breast	**3**
TCC Bladder	**1**
Adenoid cystic carcinoma	**1**

*Stage II, III and IV resected

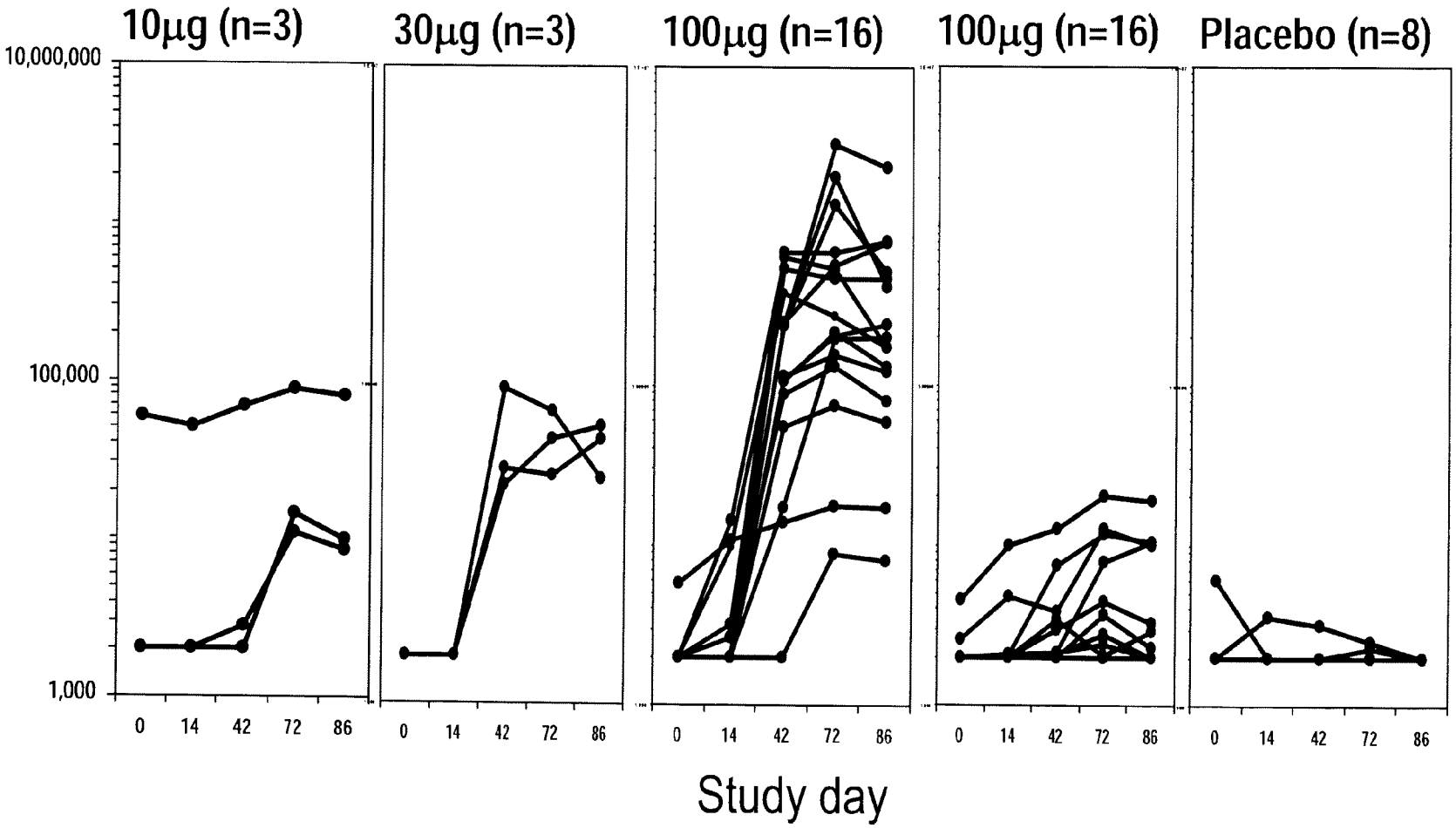
Toxicity

- NY-ESO-1 ISCOM® was well tolerated
- Most adverse events were grade 1 or 2
- Grade 3 toxicities: injection site pain in 3/46
- Common grade 2 toxicities (2 or more patients)
  - Injection site pain
  - Fever
  - Myalgia
  - Headache
  - Flu-like symptoms

Assays

- DTH using NY-ESO-1 protein alone
- Antibody (capture ELISA)
- CD8+ T cells
  - Tetramer: SLLMWITQC
  - Cytospot: γIFN producing CD8+T Cells)
- Assays under development
  - *CD4+ T cells (DC & protein: cytokine secretion)*
  - *Class I epitopes - non HLA-A2*

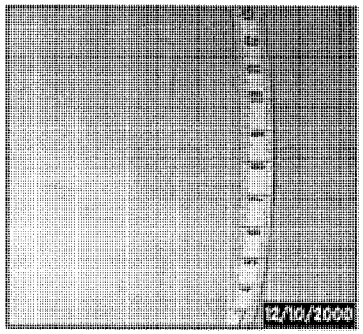
Antibody titre by cohort



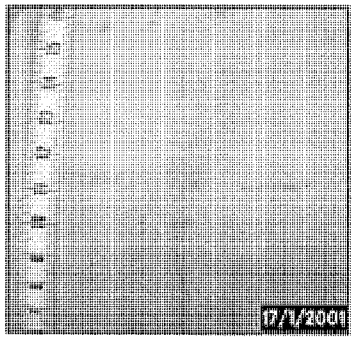
Delayed-type Hypersensitivity: 1 μg protein

Dose B (A2+) : 30μg
NY-ESO-1-ISCOM®

105/J-M



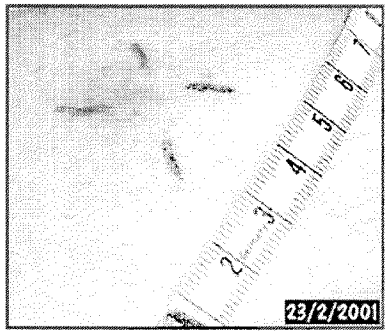
PRE
Erythema = 13
Induration = 14



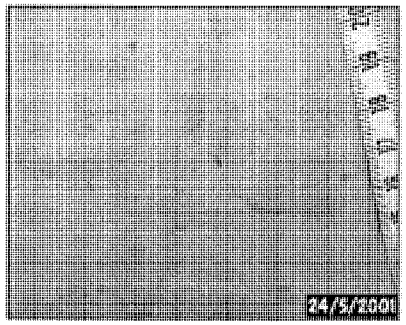
Day 86
Erythema = 60
Induration = 12

Dose C (A2-) : 100μg
NY-ESO-1-ISCOM® / Placebo

115/N-F



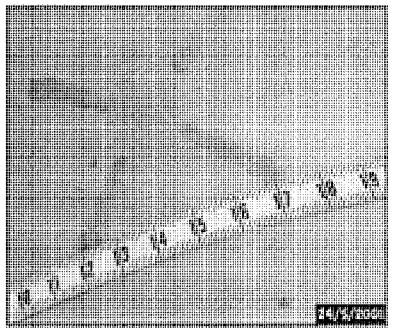
PRE
Erythema = 25
Induration = 4



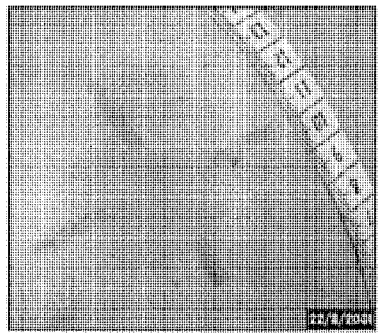
Day 86
Erythema = 50
Induration = 34

Dose C (A2+) : 100μg
NY-ESO-1-ISCOM® / Placebo

126/KLE



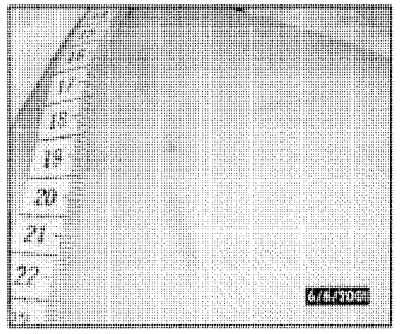
PRE
Erythema = 15
Induration = 3



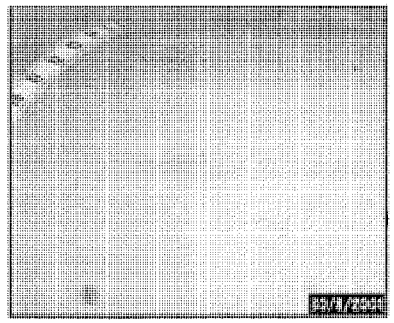
Day 86
Erythema = 60
Induration = 25

Dose D (A2-) : 100μg
NY-ESO-1 Protein / Placebo

127/JSM



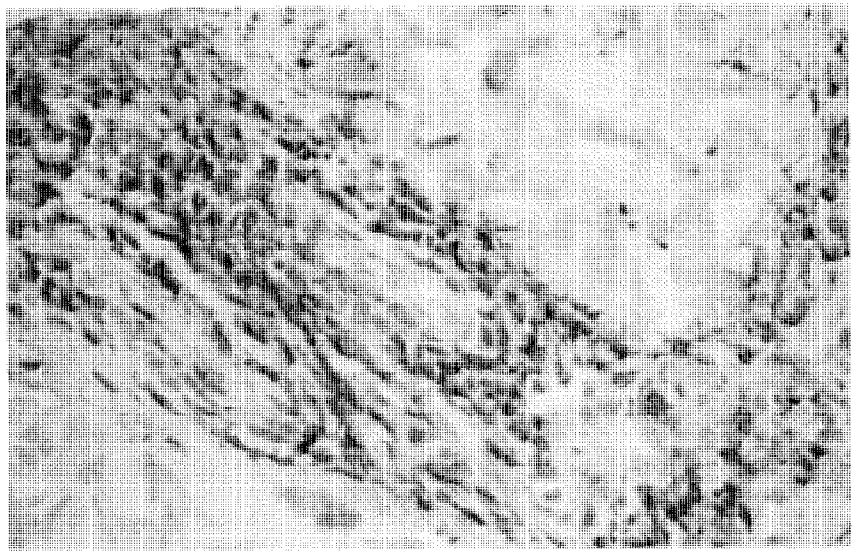
PRE
Erythema = 2
Induration = 0



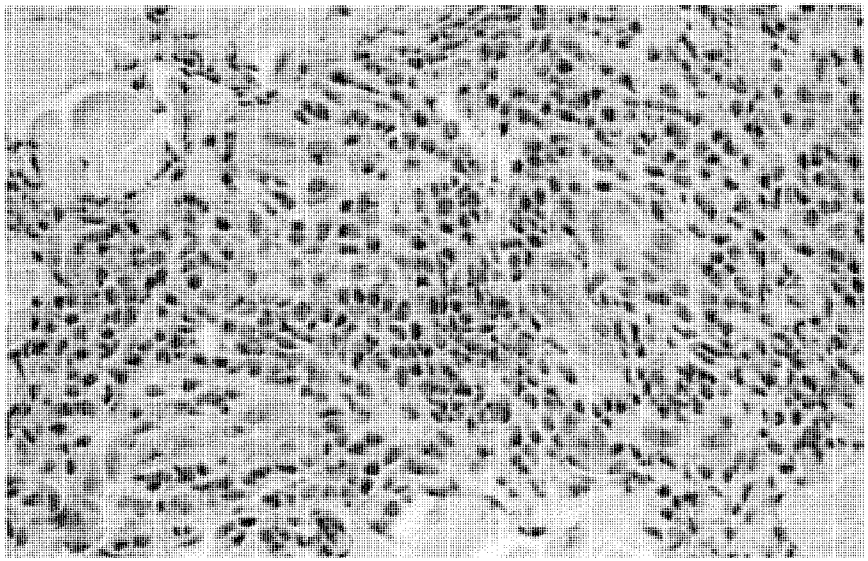
Day 86
Erythema = 12
Induration = 0

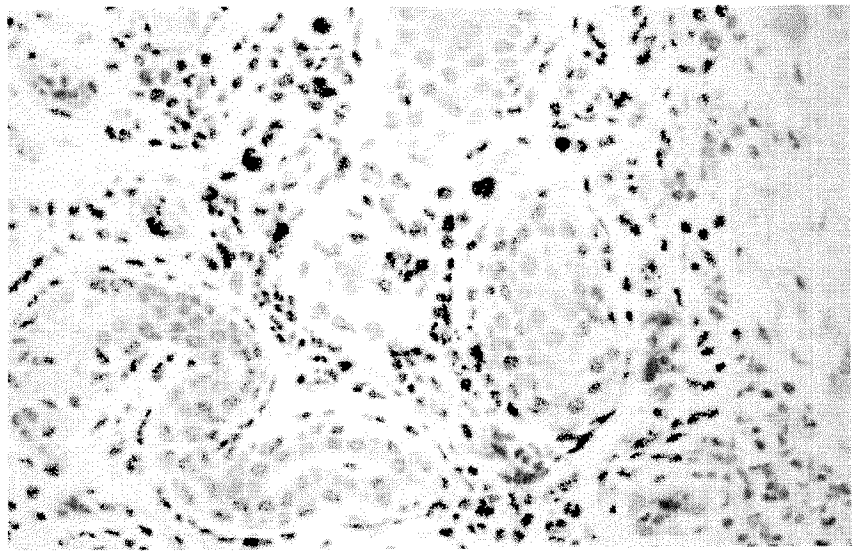
DTH response to 1mg NY-ESO-1 protein

CD4



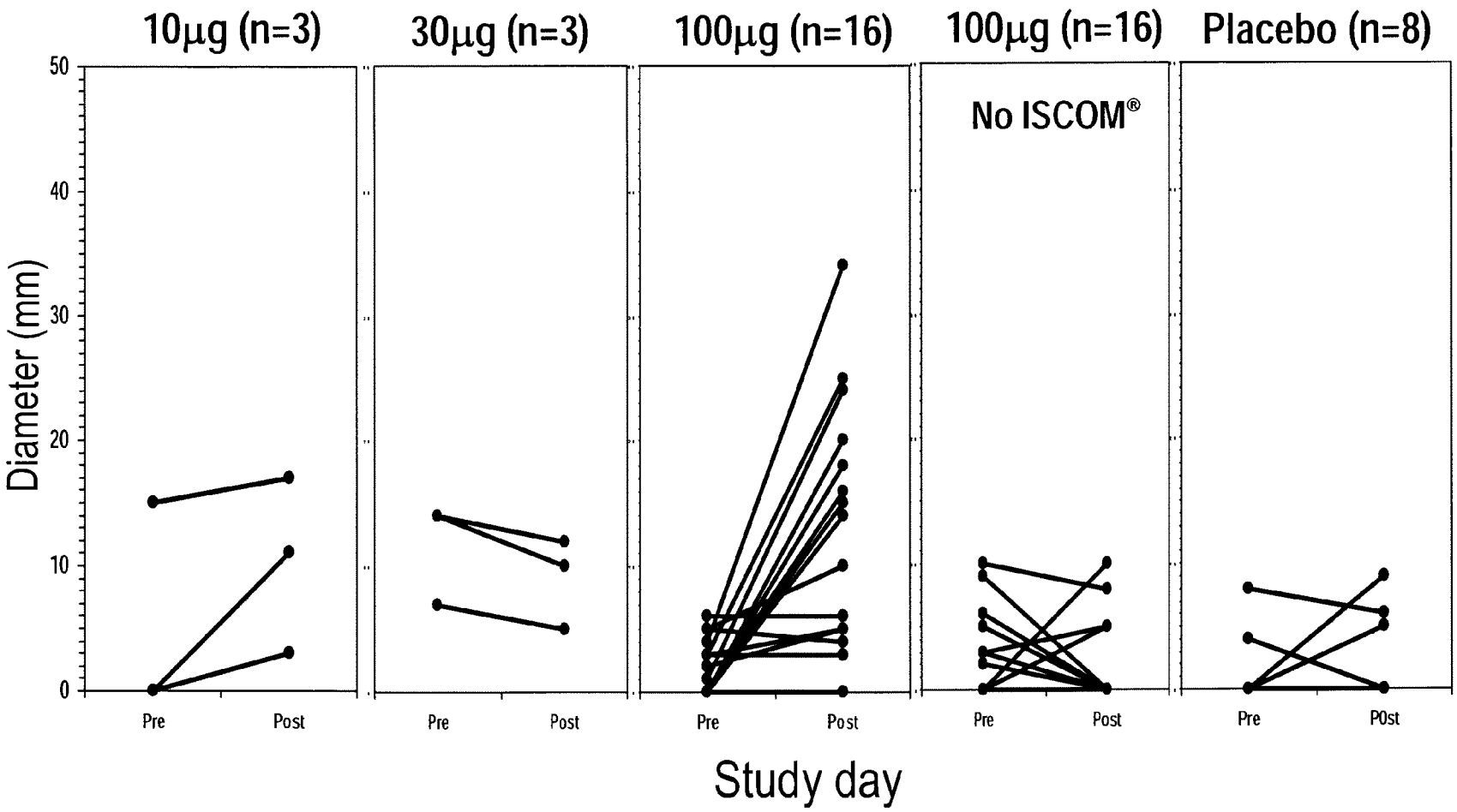
H&E





CD8

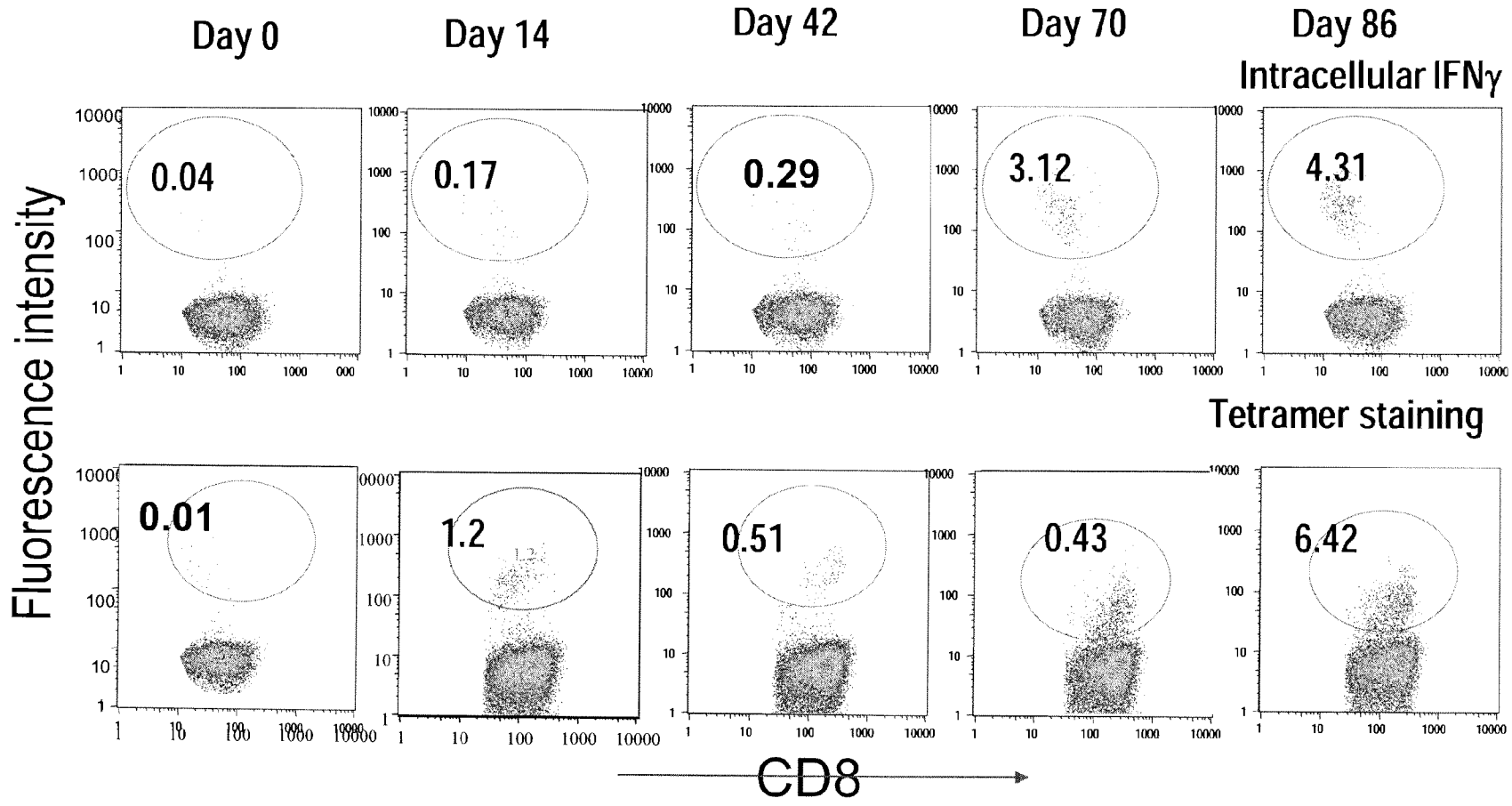
DTH Induration by cohort



Cytospot assay method

- Blood collected, ficolled and frozen
- Batch assayed on one day
- PBMC activated with peptide in bulk culture using peptides (0.5mM) in the presence of 250mM TCEP (a reducing agent for breaking the disulfide bounds formed between ESO peptides).
- An internal control was established to enable data comparison for multi trial time points---EBV BMLF1.280-288-specific CTL
- Cells were expanded for 7 days
- The CTL were activated or T2 cells pulsed +/- peptides
- BFA was directly added and the assay was harvested at 4 hours.
- Cytospot +Tetramer analyses were performed
- Controls:
  - +ve control for NY-ESO-1: Patient with known ESO1a and ESO1b response
  - Control for non-specific immune activation: EBV
  - Control for non-specific activation by T2 cells: Non-pulsed T2 cells
  - Control for non-specific peptide: MAGE 3

T- cell response: γIFN production
HLA A2+ pt (peptide SLLMWITQC)



Summary Immunological Data

DTH (doubling or greater of induration)

A	B	C	D	Placebo
1/3	0/3	11/16	2/16	2/8

Antibody

A	B	C	D	Placebo
3/3	3/3	16/16	4/16	0/8

Cytospot & Tetramer

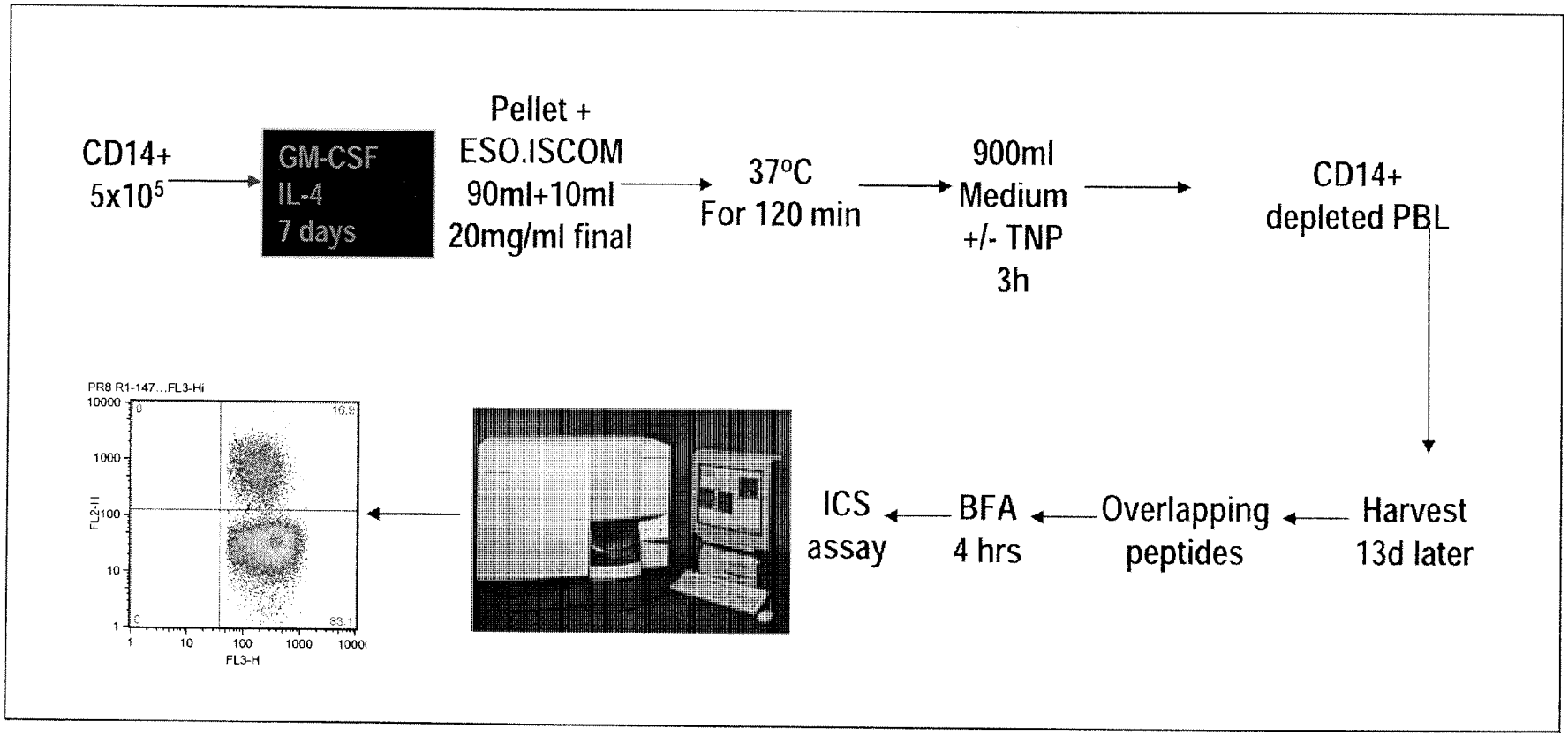
A	B	C	D	Placebo
1/3	0/3	3/8	1/8	0/4

Conclusions

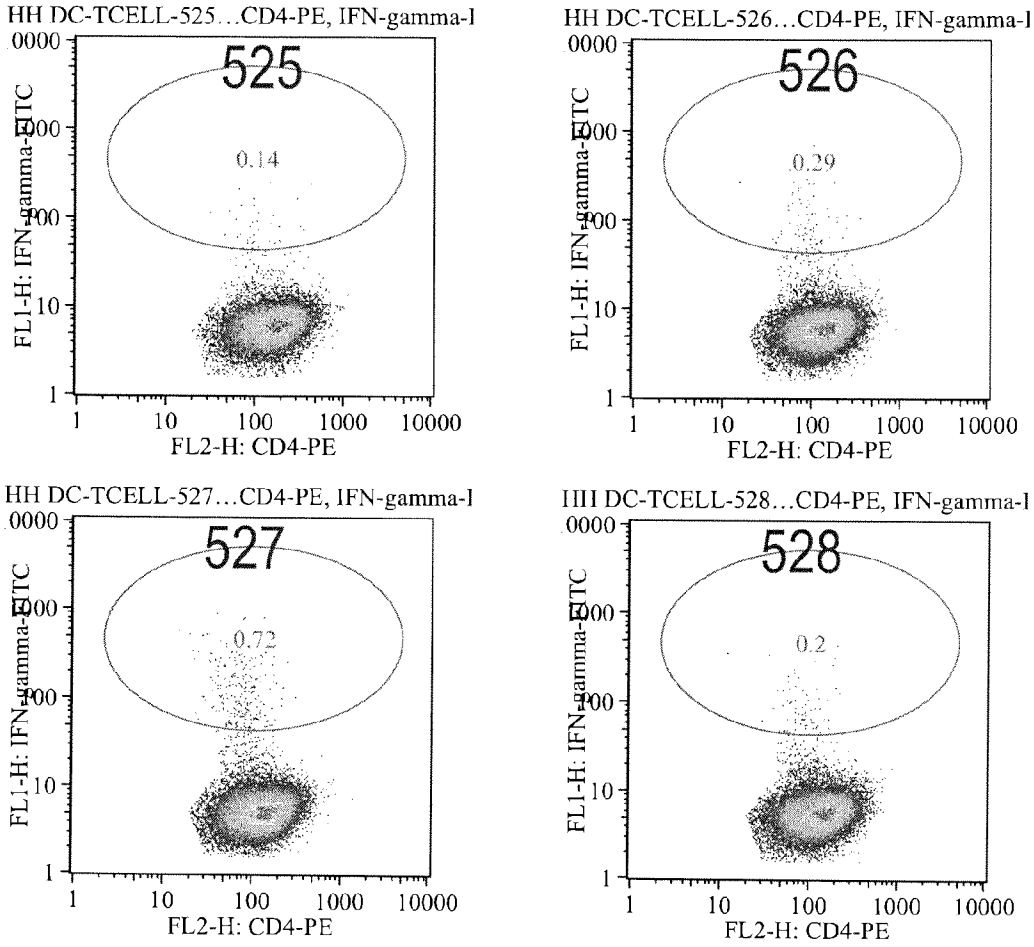
- NY-ESO-1 ISCOMs vaccinations were safely tolerated
- NY-ESO-1 ISCOMs generated both humoral & cellular responses
- ISCOM adjuvant generated superior DTH and antibody responses
- Cytospot assay in HLA A2+ve patients: positive in 1 level A pt (with prior Ab response), 3/8 level C patients and 1/8 level D patients.
- These responses were seen in patients with and without pre-existing antibody titres
- There was a good correlation between tetramer & cytospot data
- Is there evidence of immune response to other epitopes?

Identification of response to other epitopes
Class I & Class II

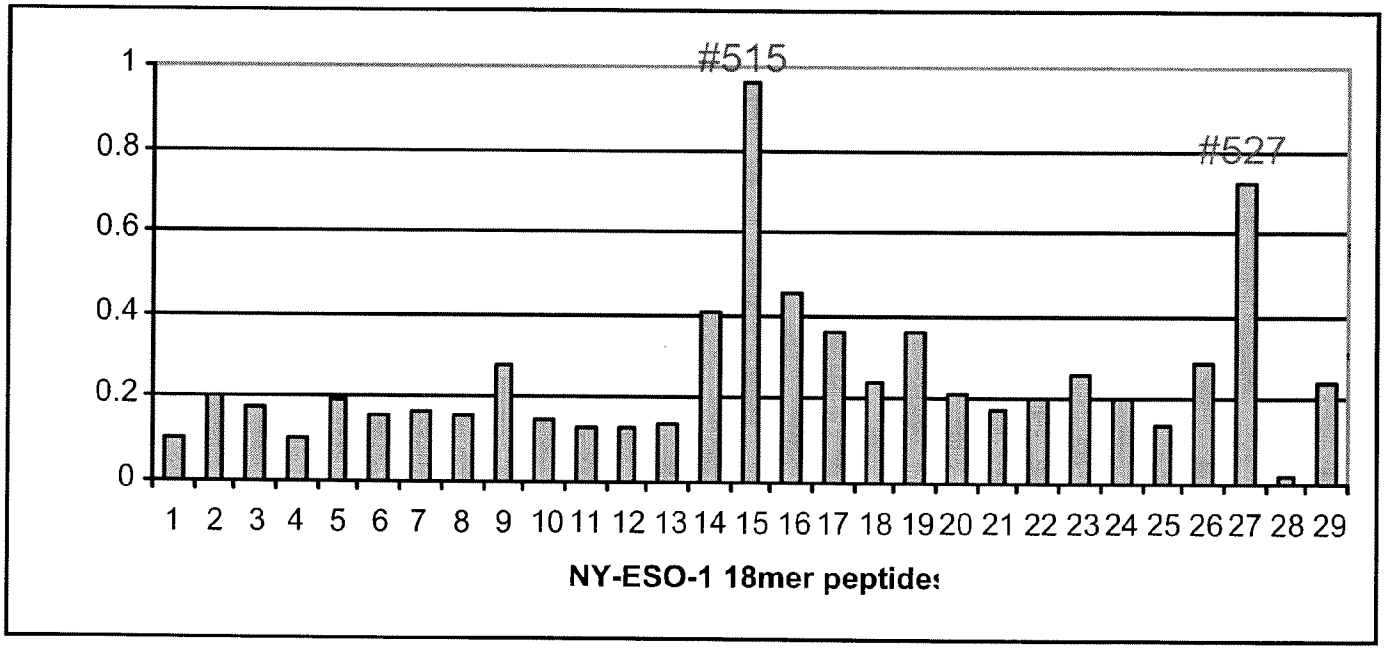
Generation of NY-ESO-1 Specific T cells Using Tumor-Ag-loaded Autologous-DC



Pt 107 T cells generated with DC+ISCOM/NY-ESO-1 and screened with NY-ESO-1 18mer on day 13 after culture



T cells generated with DC+ISCOM/NY-ESO-1 and screened with 18mer peptides at day 13 after culture

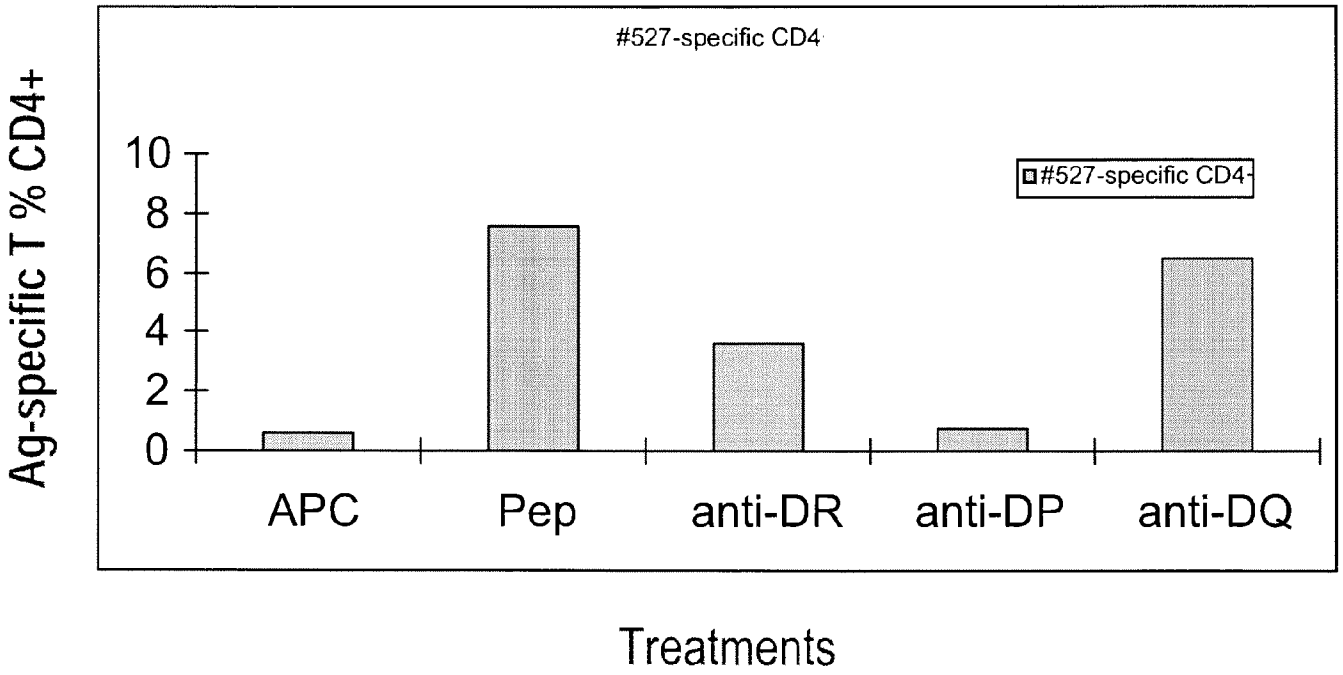


Further characterisation of DC generated CD4 T cells

- Lines & clones established
- Antibodies
  - Anti DR, DP, DQ
- LCL lines
  - LCL auto: DR1, DR2, DP4
  - LCL 9080: DR1, ---, ---
  - LCL 9014: ---, DR2, ---
  - LCL T291: ---, DR2, DP4
  - LCL T282: ---, ---, DP4
- Tumor lines
  - NW38: DR1, ---, ---, NY-ESO-1(+)
  - LAR1a: ---, DR2, ---, NY-ESO-1(+)
  - SK-Mel 37: ---, ---, ---, NY-ESO-1(+)

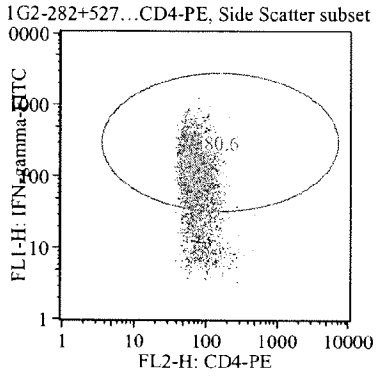
#527-specific CD4+ T cells are DP restricted

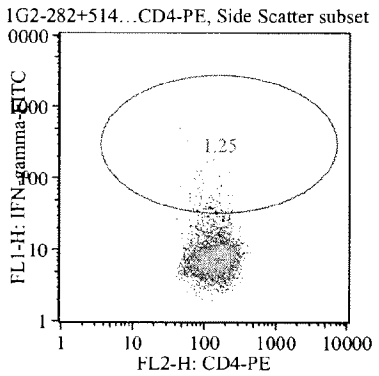
(DC stimulated then #527-pulsed BCL stimulated 2x)



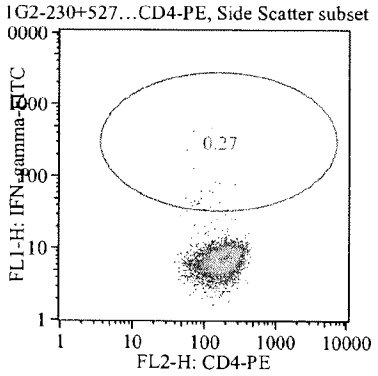
#527-specific T cells are DP4 restricted

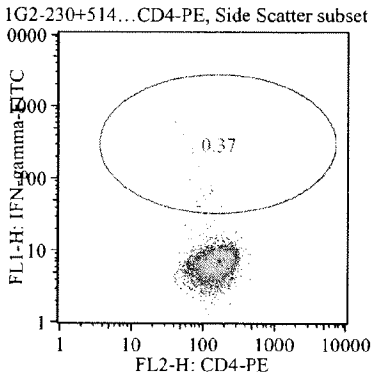
DP4+ LCL (282)





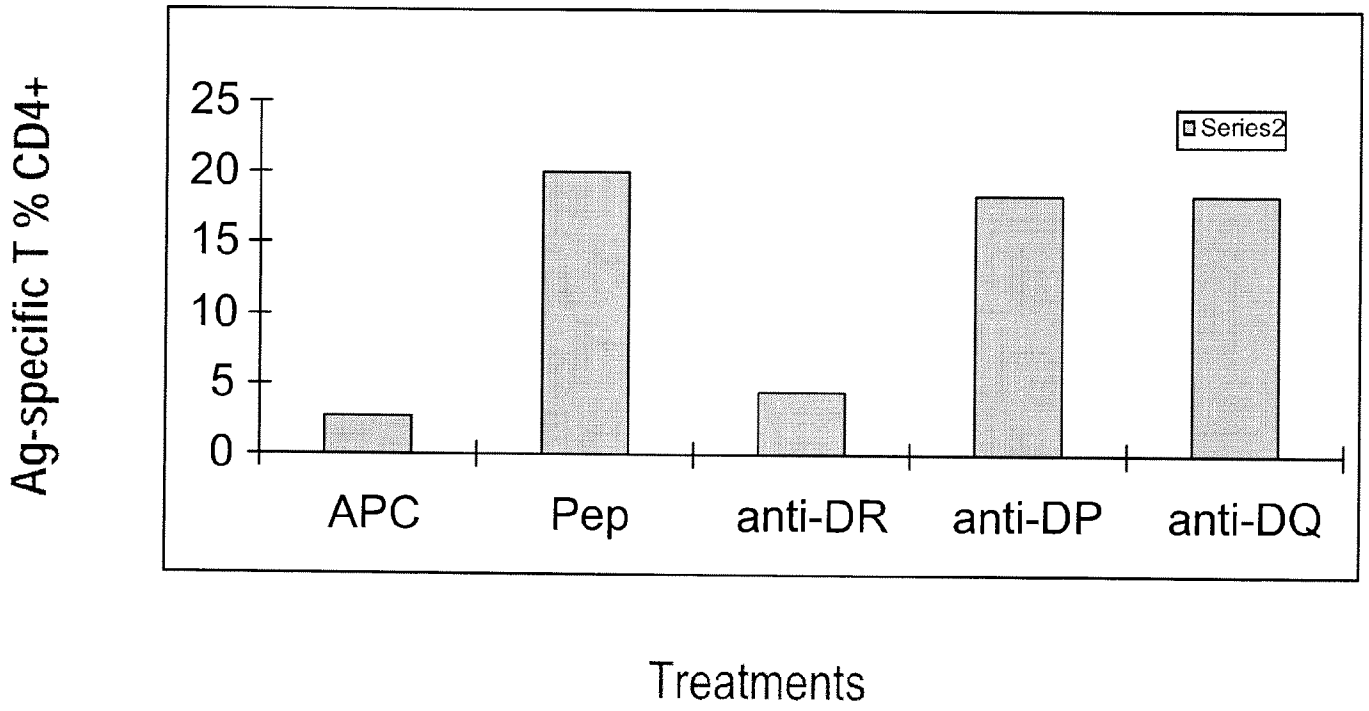
DP4- LCL (230)



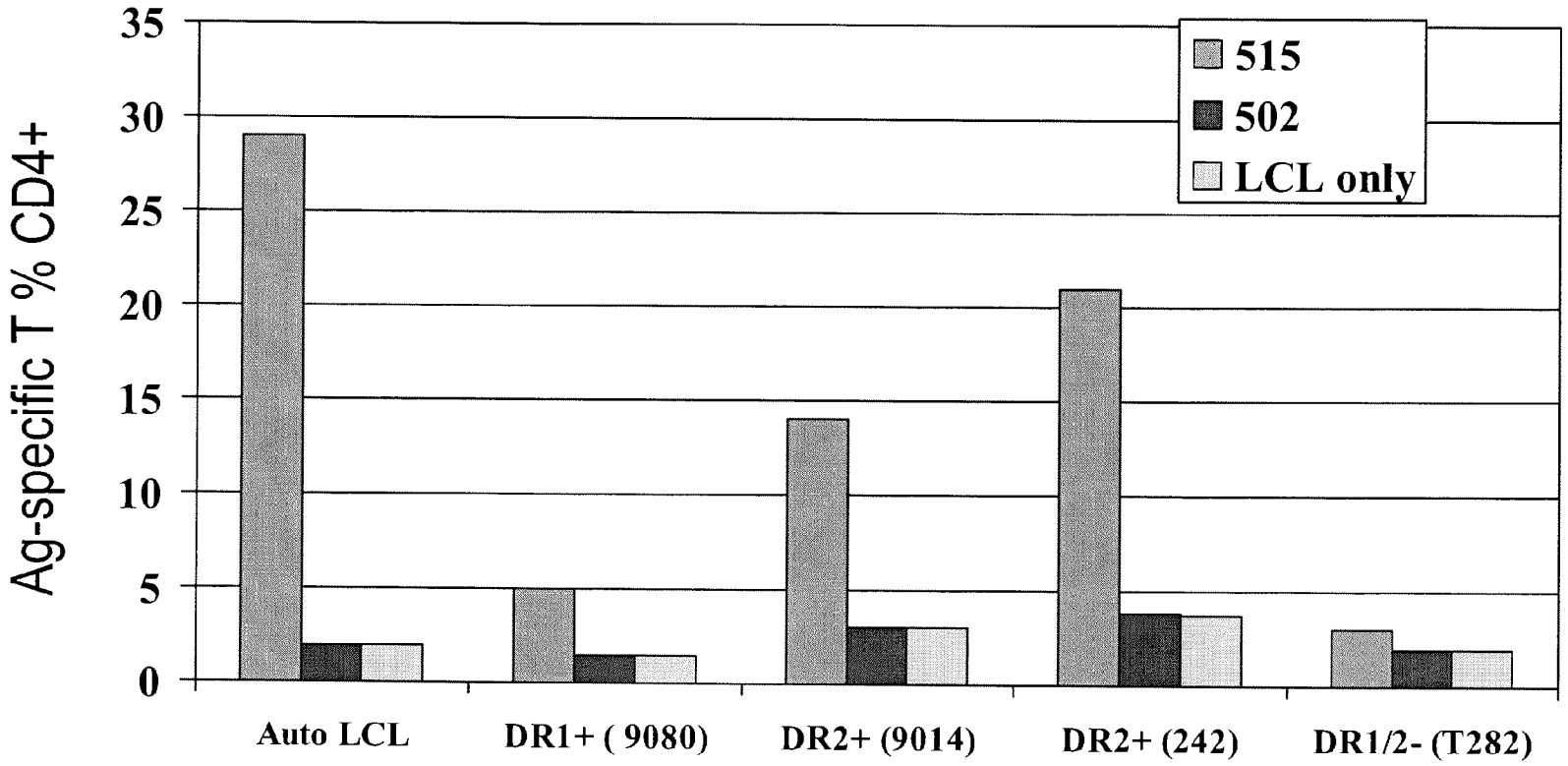


#515-specific CD4+ T cells are DR restricted

(DC stimulated then #515-pulsed BCL stimulated 2x)

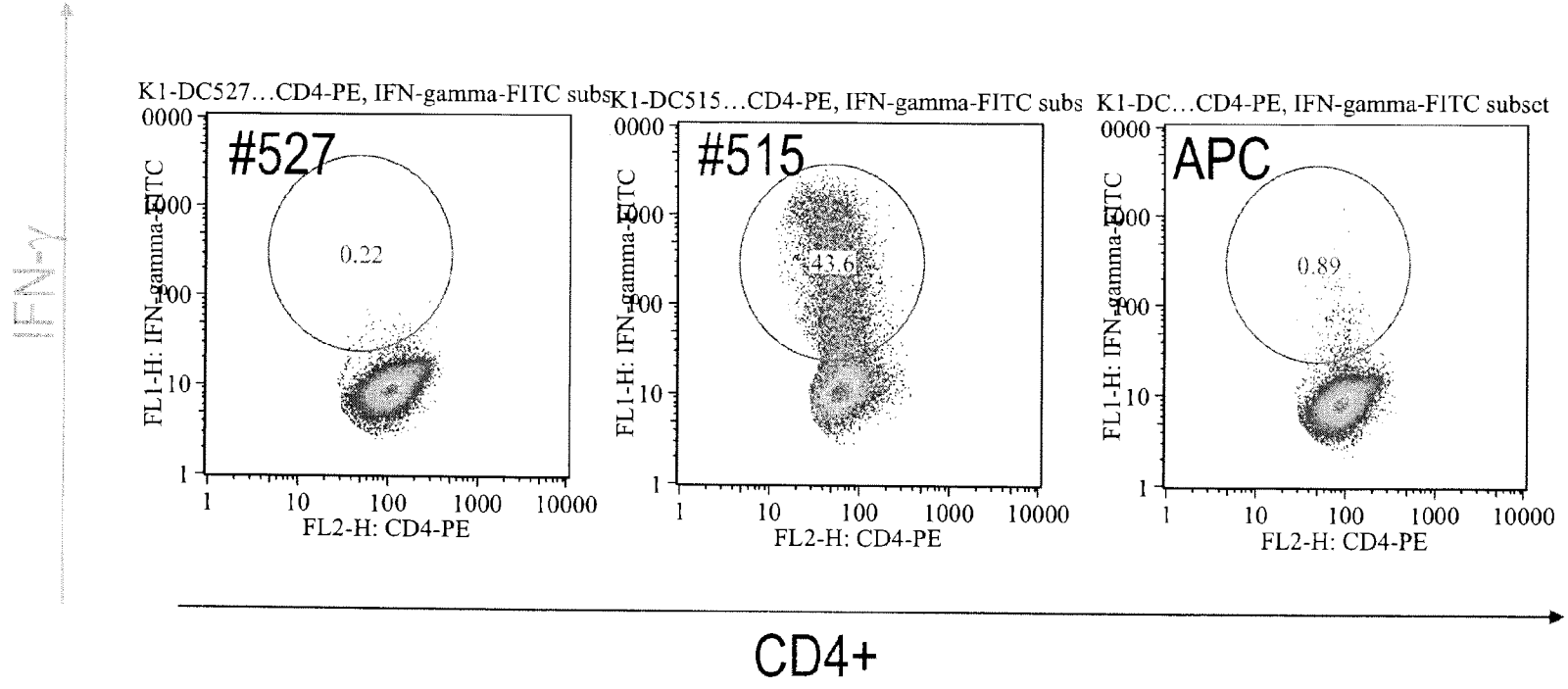


DR restriction of #515-specific T cells
(#515 2xstimulations)

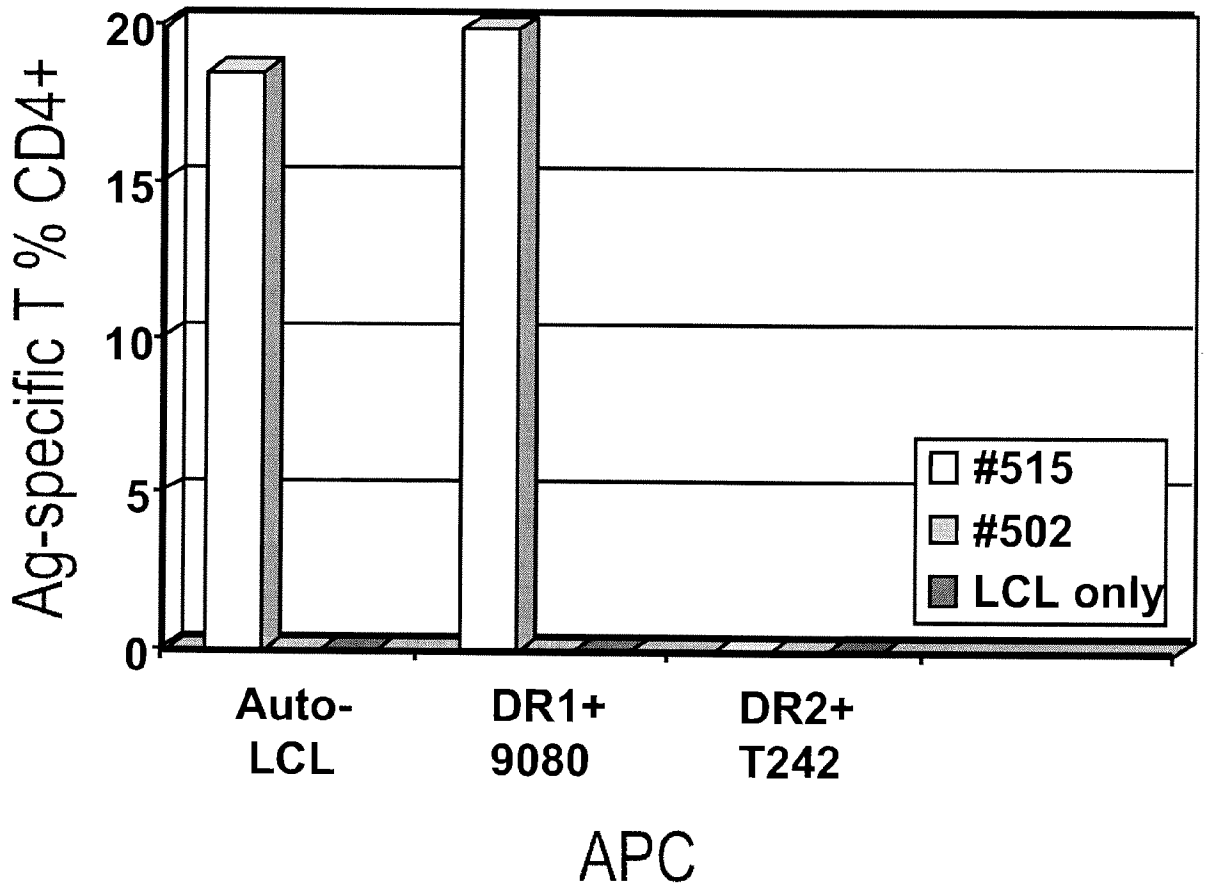


#515-specific T clone K1

generated with #515-pulsed DC



T clone K1 is DR1-restricted



Summary

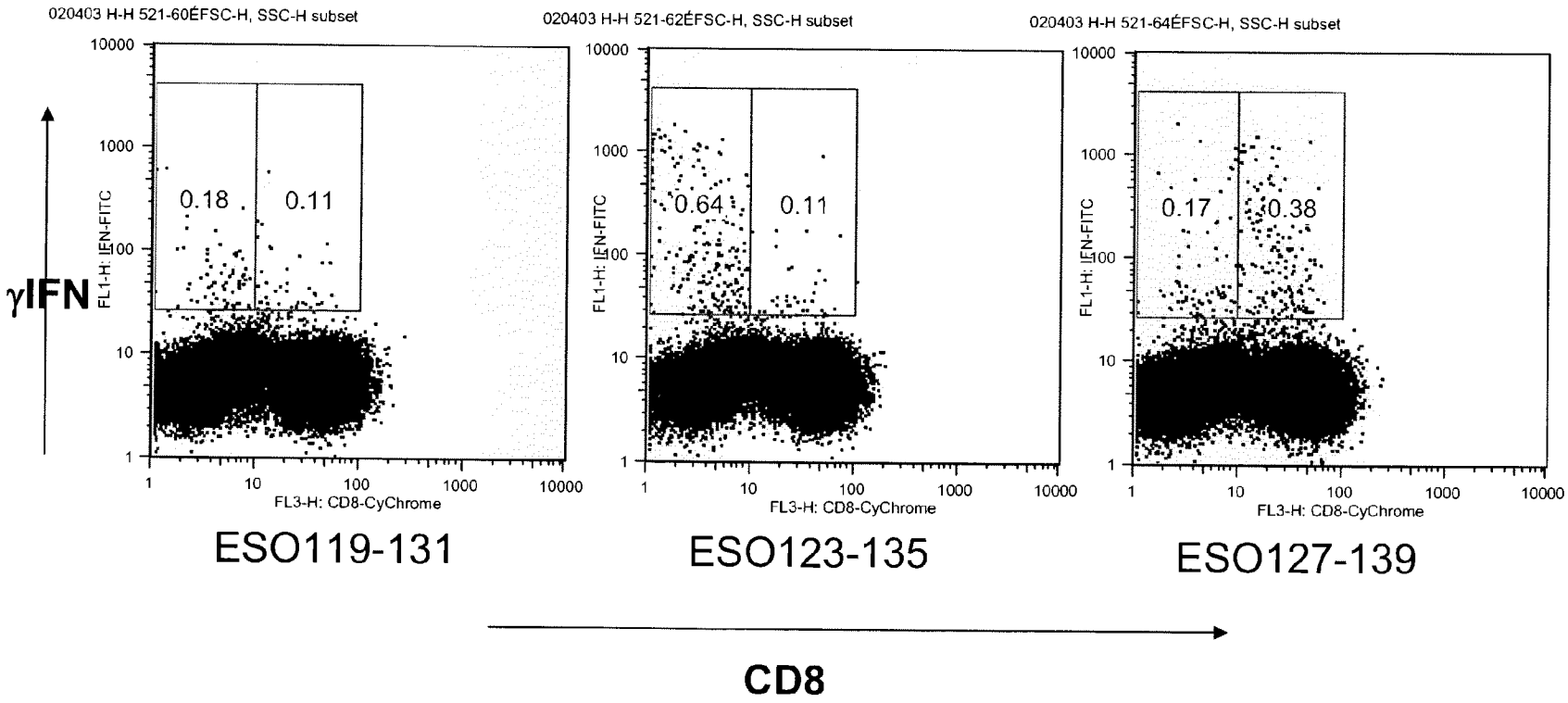
- Response identified to 3 probable Class II peptides
- Restricted by
  - DP4 (previously reported)
  - DR1
  - DR2
- Minumum epitope is being determined

Simplified method for screening for CD4/CD8 response to ESO/ISCOM vaccine against peptide panels

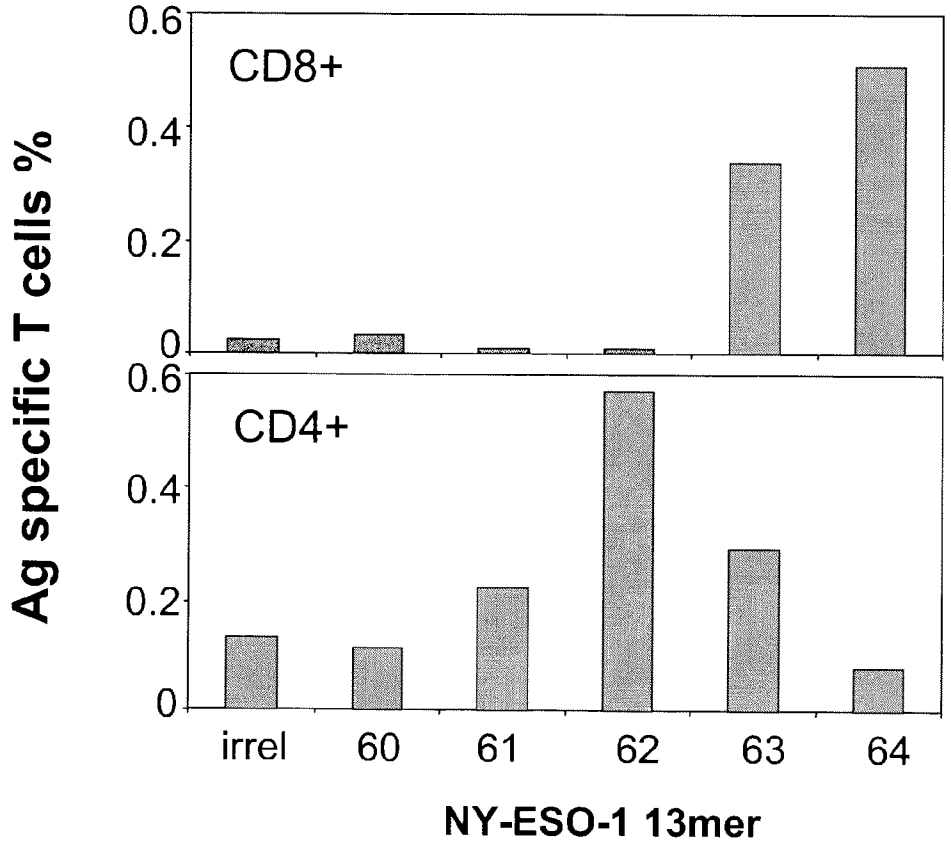
Method

1. Frozen PBMC Day 86
2. Autologous cells used as APC
3. Bulk cultures stimulated with NY-ESO-1 18mer peptides
4. On day 7, cultures screened for intracellular cytokine staining (ICS) for IFNγ against panel of 18mer peptides pulsed onto autologous PBMC.
5. Day 9: Positive cultures were further tested against a panel of shorter overlapping peptides (13mers)
6. Day 17: Confirmation assay: ICS performed again using the same 13mer peptides
7. All ICS were triple colour for CD8(CyCh), CD4(PE) and γIFN-FITC.

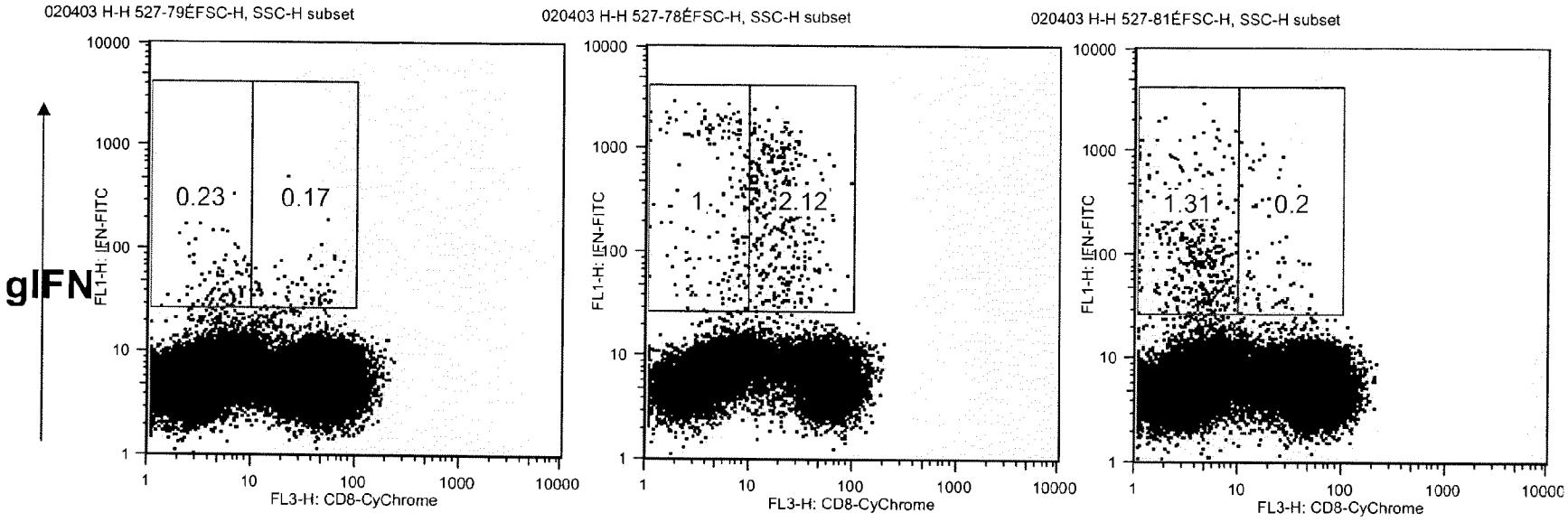
Pt 107 CD4+CD8 Bulk culture screening #521 (ESO121-138) stimulated T cells



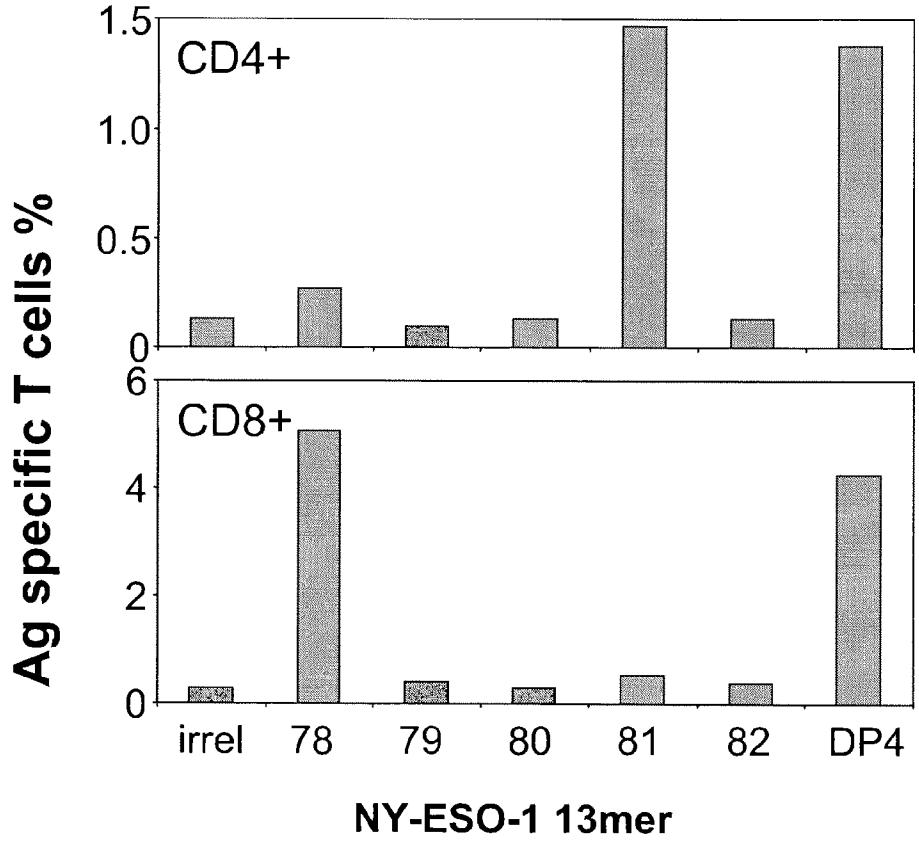
Pt 107 CD4+CD8 Bulk culture screening #521 (ESO121-138) stimulated T cells



Pt 107 CD4+CD8 Bulk culture screening #527 (ESO157-174) stimulated T cells



Pt 107 CD4+CD8 Bulk culture screening #527 (ESO157-174) stimulated T cells



ESO-1(139-156)	AADHRQLQLSISSCLQQL	(peptide #524)
ESO-1(145-162)	LQLSISSCLQQLSLLMWI	(peptide #525)
ESO-1(151-168)	SCLQQLSLLMWITQCFLP	(peptide #526)
ESO-1(157-174)	SLLMWITQCFLPVFLAQP	(peptide #527)
ESO-1(163-180)	TQCFLPVFLAQPPSGQRR	(peptide #528)

Previously described Epitopes

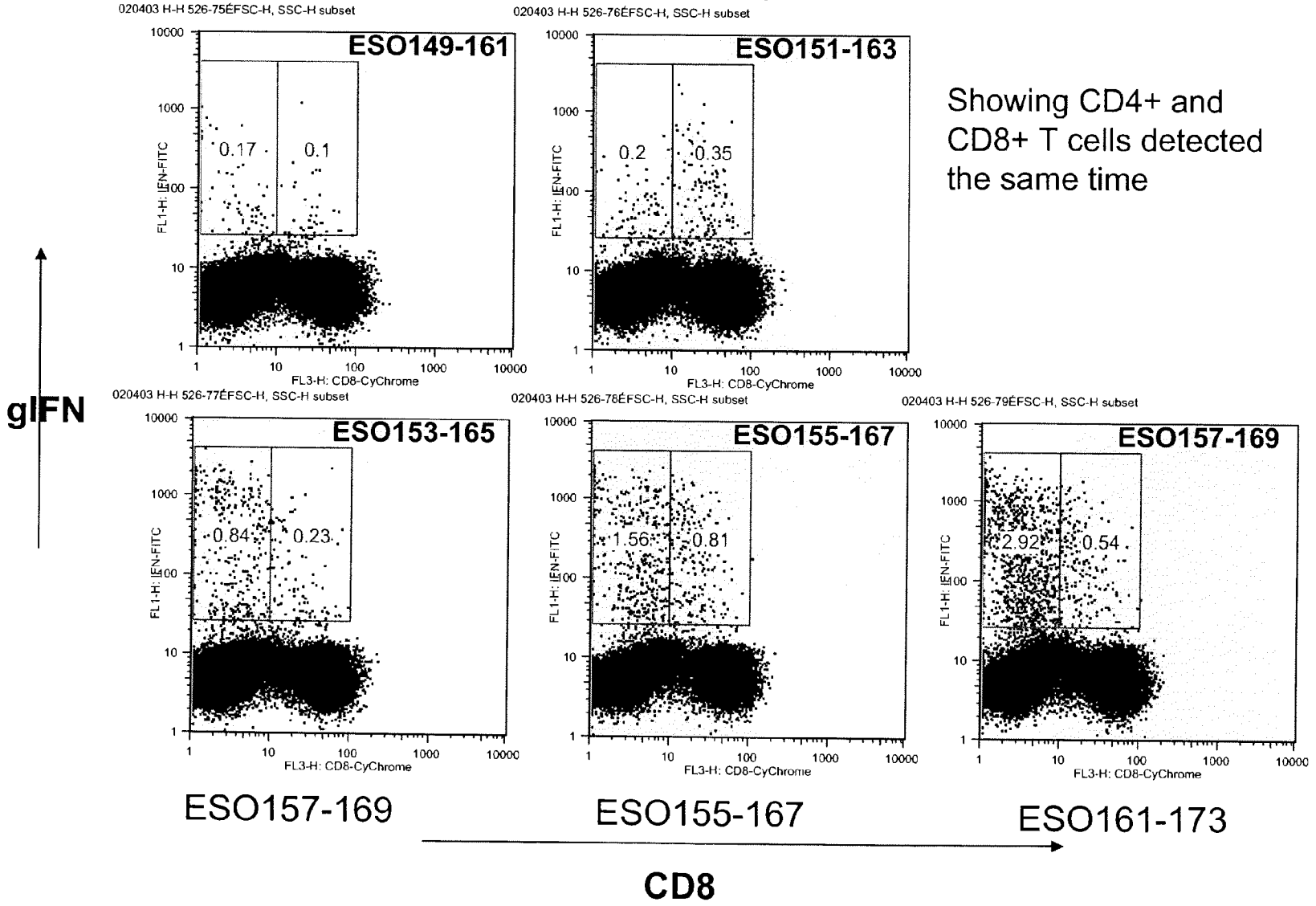
HLA DP4

SLLMWITQCFLPVF

HLA-A2

ESO-1a(157-167)	SLLMWITQCFL
ESO-1b(157-165)	SLLMWITQC or SLLMWITQV
ESO-1c(155-163)	QLSLLMWIT
ESO-1d(159-162)	LLMWITQCF

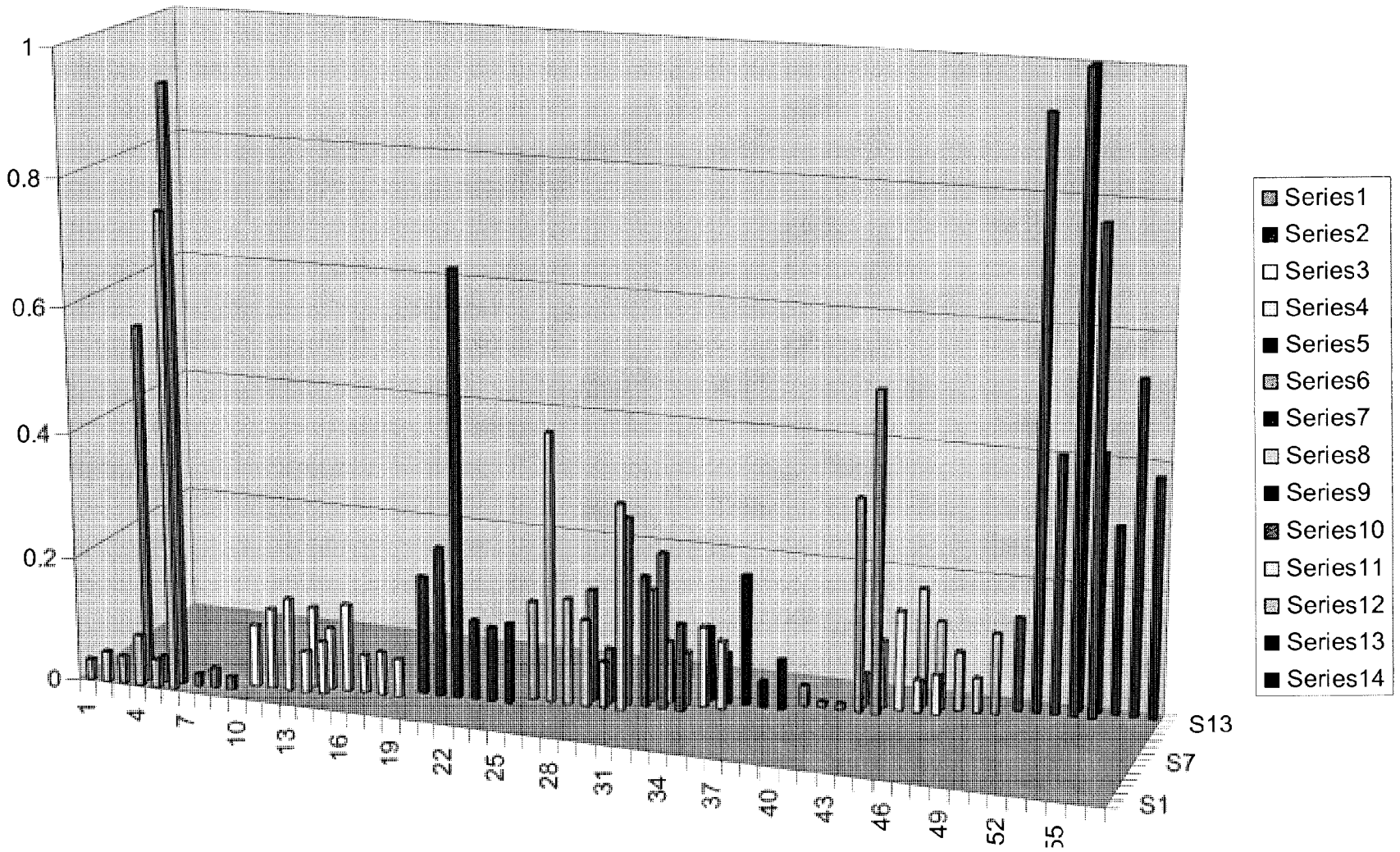
Pt 107 CD4+CD8 Bulk culture screening #526 (ESO151-168) stimulated



Showing CD4+ and CD8+ T cells detected the same time

Pt 107

HLA typing: A1, A2, B8, B27, Bw4, Bw6, C*0102, C*07011/012/06, DRB1*0101 (DR1), DRB1*1501 (DR15), DPB1*0401 (DP4), DQB1*0501 (DQ5), DQB1*0602 (DQ6).



Immunology Conclusions

- Screening for immune reactivity has been successful using a simplified methods with autologous PBMC & panels of overlapping peptides
- Ongoing work will define minimal epitopes and HLA class I and II restriction for each
- For patients tested to date there is clear evidence of a broad-based CD4 and C8 cellular immune response against NY-ESO-1 in addition to antibody responses
- We have reduced concern that contaminating bacterial protein may have dominated immune responses to this vaccine
- PBMC from Pts in this trial should make it possible to map the majority of NY-ESO-1MHC I & II epitopes.

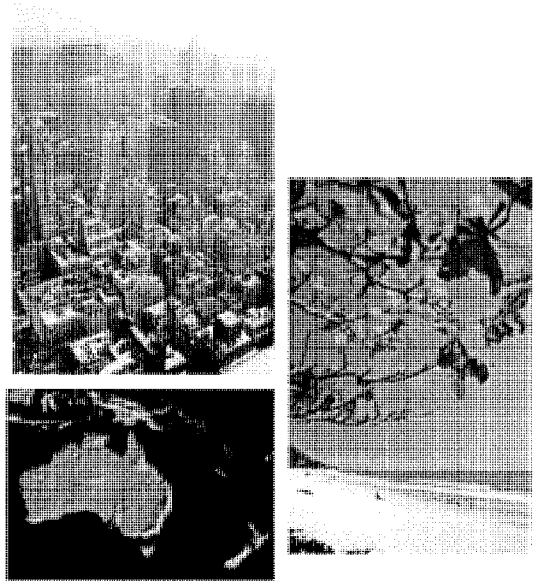
Future Directions for NY-ESO-1 ISCOM Vaccine

Clinical responses to vaccination need to be optimised.

- Lab studies
  - Further studies to map epitopes will make it possible to
    - Investigate impact of Class II determinants on class I response
    - Investigate immunodominance in a human cancer antigen system
- Clinical Directions:
  - Optimization of the vaccine
    - Route, schedule etc
    - Other (cytokines, anti-CTLA4)
  - Evaluate clinical impact
    - Patients with evaluable disease
      - What are the determinants of clinical response?
      - Prospectively identify potential clinical responders
    - Adjuvant therapy of NY-ESO-1 +ve tumours

Future Directions

- Optimising clinical strategies
- Building collaborative networks
  - Ludwig Institute International Trials Program
  - Cancer Vaccine Collaborative (New York)
    - MSKCC, Cornell, NYU, Sinai, Columbia, Roswell Park
  - Cancer Vaccine Collaborative (South Pacific)



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